CHANGES IN OLFACTORY SENSITIVITY

WITH AGE

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Seow Yi Xin

25 May 2015

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TABLE OF CONTENTS

SUMMARY

This study was centred on understanding the changes in olfactory functions with age. Without an existing olfactory test to assess identification proficiency and detection thresholds for a set of single odourants, the Specific Sensitivity Test was adapted from the Sniffin' Sticks test and developed with odourants of high familiarity to the Asian population. The test was validated using standard olfactory tests with 20 Singaporeans and Singapore Permanent Residents (PRs) of Chinese ethnicity, aged 21-80 years, and showed good reproducibility. The Specific Sensitivity Test was subsequently extended to 281 Singaporeans and PRs of Chinese ethnicity and the same age range.

Results collected from subjects across all adult age groups demonstrated heterogeneous loss of olfactory function with age. Specifically, the onset and extent of loss in both identification and detection thresholds of odourants with age varies between odourants. Subjects in their 70s only detected rose-like phenylethyl alcohol at 179 times the concentration detected by subjects in the 20s, while thresholds for onion-like 2-methyltetrahydrofuranthiol only differed by 3 times between the age groups. In addition, identification rates for phenylethyl alcohol were negatively correlated with age throughout adult life, falling from 92.1 % in the 20s to 81.6 % and 54.5 % in the 40s and 70s, respectively, while mushroom-like 1-octen-3-ol was equally identified by subjects across all ages, with identification rates ranging between 63.6 % to 76.3 %. Our results demonstrated the girth of differentiated olfactory loss due to normal ageing,

which potentially affect overall perception and preferences of odour mixtures with age.

The relationship between identification ability and threshold sensitivity of the same odourants was explored for the first time in literature. Age was a mediating factor in the interaction between the two olfactory measures, especially for odourants of low molecular weight ($M_r \leq 122.2$), such as butyric acid ($M_r = 88.1$; $p = 0.002$) and acetyl pyrazine ($M_r = 122.1$; $p < 0.001$). The findings have implications for the potential of olfactory training to compensate or slow down the rate of loss in olfactory function.

To compensate for differentiated olfactory loss and increase palatability of foods consumed by the elderly, the flavour modification method was used to adjust ratios of odour groups in mango and coffee flavours before the modified flavours were applied into beverage formulations. As expected, the flavour enhancement method commonly undertaken by previous research groups to increase palatability of foods for the elderly did not yield favourable hedonics in our study. Our findings also indicated that the preconceived concept of the flavours used in the study was found to be a strong influence for hedonic ratings. While the modified flavours were rated favourably by the elderly consumers, they preferred the original flavours with flavour profiles most closely resembling the real fruit and beverage. Thus, the flavour modification method showed potential in compensation for olfactory loss in the elderly, but consumer expectations of flavours need to be considered before implementation of method and consumer sensory evaluation.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASSIT	Adapted Specific Sensitivity Identification Test
ASSTT	Adapted Specific Sensitivity Threshold Test
ATT10	Adapted 10-Item Threshold Test
FDV	Final dilution value
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GC-O	Gas Chromatography-Olfactometry
GCO-MS	Gas Chromatography-Olfactometry-Mass Spectrometry
OFC	Orbitofrontal cortex
OR	Olfactory receptor
OSN	Olfactory sensory neuron
PR	Permanent Resident
SSSIT	Standard Sniffin' Sticks Identification Test
SSSTT	Standard Sniffin' Sticks Threshold Test

LIST OF CONFERENCES ATTENDED

1. Seow, Y.-X., Ong, P.K.C., Huang, D. (2014). Odourant-specific loss of olfactory sensitivity with age. *SenseAsia 2014*, Singapore on 11-13 May 2014.
2. Seow, Y.-X., Ong, P.K.C., Huang, D. (2014). Heterogeneous loss of olfactory sensitivity with age. *14th Weurman Flavour Research Symposium*, Cambridge, UK on 15-19 September 2014.
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CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1.1. Olfaction

Olfaction refers to the sensation that arises from the stimulation of olfactory receptors/neurons located in the nose (Pinto, 2011). Odourants, or odour molecules, stimulate olfactory receptors of the olfactory epithelium in the nose (Buck & Axel, 1991). Upon binding with olfactory receptor proteins, olfactory sensory neurons in the epithelium transmit neuronal signals to the olfactory bulb, where it forms spatial images by activation of the glomeruli (Pinto, 2011; Shepherd, 2006). Odour images are then projected to the olfactory cortex in the orbitofrontal cortex (OFC), where the integration of interactions with other areas of the brain, such as the amygdala, areas of the OFC, and hippocampus, form odour perception (Shepherd, 2006).

The spatial images elicited by odourants are complex and different, but overlapping, and extents of activation are dependent on odourant intensity, thus contributing to humans' ability to discriminate between more than one trillion odours (Bushdid, Magnasco, Vosshall, & Keller, 2014). The human

olfactory system is also remarkably sensitive. Humans are able to detect odourants at concentrations ranging from parts per thousand to parts per billion. While alcohols like ethanol are detected at about 100 ppm, potent odourants such as methylthiol are detectable at 0.02 ppb in water at 20 °C (Belitz et al., 2009).

Olfaction plays an important role in human food perception and feeding. The integration of odour perception and the other sensory modalities, taste, hearing, touch, and vision, in the brain lead to motivation and cravings for food consumption (Shepherd, 2006; Doty & Kamath, 2014). In addition, olfaction also impacts habits, social interactions, behaviour, emotions, and moods (Miwa et al., 2001; Willander & Larsson, 2007; Chrea et al., 2009).

1.2. Olfactory Measures and Testing

To determine olfactory ability, psychophysical tests for the measure of olfactory functions include the ability to detect, discriminate, and identify odours. In odour threshold testing, the detection threshold is regarded as the lowest concentration of the stimulus, i.e. an odour, where a presence is detected. Odour discrimination is the ability to make a distinction between two different odours at suprathreshold and similar intensities. Finally, odour identification refers to the ability to correctly label an odour at suprathreshold concentration, i.e. above detection threshold, verbally, and thus dependent on previous associations.

Various olfactory tests utilize one or more of these measurements to evaluate olfactory function. Olfactory tests have been applied in clinical

settings to assess olfactory deficits with diseases, such as Parkinson's and Alzheimer's diseases (Makowska, Kloszewska, Grabowska, Szatkowska, & Rymarczyk, 2011; Peters et al., 2003; Rahayel, Frasnelli, & Joubert, 2012). In addition, olfactory tests have also been used to study changes in olfactory function by gender and age (Dudova et al., 2011; Mueller, Temmel, Quint, Reiger, & Hummel, 2002; Weinstock, Wright, & Smith, 1993; Yang, Wei, Yu, Zhang, & Liu, 2010).

Some of the olfactory diagnostic tests most commonly reported in literature are:

- Connecticut Chemosensory Clinical Research Center test (CCCRC) (Cain, Goodspeed, Gent, & Leonard, 1988),
- University of Pennsylvania Smell Identification Test (UPSIT) (Doty, Shaman, & Dann, 1984b), and the shortened version of UPSIT, Cross-Cultural Smell Identification Test (CC-SIT) (Doty, Avron, & Lee, 1996), and
- Sniffin' Sticks (Hummel, Sekinger, Wolf, Pauli, and Kobal, 1997)

These olfactory kits have been utilized to obtain normative values from the United States of America, across Europe, and in Australia (Cain, 1989; Hummel, Kobal, Gudziol, & Mackay-Sim, 2007). The kits are low cost, mobile, and require relatively short examination time for the diagnosis of olfactory function. While the Sniffin' Stick presents subjects with a liquid odour-filled felt tipped pen for odour delivery, UPSIT and CC-SIT uses Microfragrance^{TM} "scratch 'n' sniff" labels, and CCCRC utilises squeeze bottles filled with test solutions.

In Asia, Saito et al. (2006) developed an olfactory performance test kit to overcome the cultural differences in familiarity with the odours used in the tests above. The Odor Stick Identification Test for the Japanese (OSIT-J) utilises a stick-type semi-solid cream with odours familiar to the Japanese population. The semi-solid cream is shaped into a lipstick, twisted in and out of the case, and used to draw on a thin paraffin paper, rubbed to release microencapsulated volatiles, then presented to the subject for sniffing.

Other research groups have overcome the socioeconomic and cultural factors by adapting the tests using odours which are cross-cultural or specific to a population. Two separate research groups, in Italy and Brazil, replaced a few odours of the UPSIT and developed versions of the UPSIT to more accurately evaluate olfactory functions of the Italian and Portuguese populations, respectively (Fornazieri et al., 2013; Parola & Liberini, 1999).

Of the abovementioned olfactory performance tests, the Sniffin' Sticks test is the only olfactory performance test for all three olfactory functions, identification, discrimination, and detection threshold, whilst only identification ability is tested for the rest. Yet, although the Sniffin' Sticks test was developed in Europe, several research groups outside of Europe were also able to utilize the test to obtain normative values of olfactory capability (Hummel et al., 2007; Tekeli, Altundağ, Salihoğlu, Cayönü, & Kendirli, 2013; Yuan, Lee, Lee, Lin, & Shu, 2010).

1.3. Sniffin' Sticks

The Sniffin' Sticks test, developed by Kobal et al. (1996) and accredited by the German Society of Otorhinolaryngology, is a cost-effective, reusable, and portable test kit that utilises subjects' sniffing behaviour (Laing, 1983) to provide optimum perception, and consists of three subtests for olfactory functions – odour identification, discrimination and odour threshold testing. Sniffin' Sticks have shown long shelf stability for both mixtures and single odourants at various concentrations (Kobal et al. 2000) and test-retest of the olfactory detection threshold subtest of the Sniffin' Sticks test is highly reliable even if repeated more than once a day and across a long-term period (Albrecht et al., 2008). The Sniffin' Sticks have been used in more than 100 published studies (Kobal et al., 2000; Hummel et al., 2007; Katotomichelakis et al., 2007; Yuan et al., 2010), an indication of their ease of use and accessibility.

Published normative data on the test are also available for sample populations of up to 3,282 subjects across Europe and Australia (Hummel et al., 2007), providing a database for comparison and evaluation of olfactory results, offering valuable quantitative information as a basis for reference. Therefore, the Sniffin' Stick was deemed a suitable tool for the delivery of odourants to subjects for the purpose of this project.

1.4. Gas Chromatography-Olfactometry and CharmAnalysis

Apart from olfactory performance test kits for the measure of olfactory function, Gas Chromatography-Olfactometry (GC-O) dilution analysis (Acree,

Barnard, & Cunningham, 1984) has been demonstrated to be a reproducible method for determining gas-phase odour detection thresholds (Marin, Acree, & Barnard, 1988).

The GC-O is an analytical tool that combines GC separation of volatile compounds, with the use of a human assessor to detect and evaluate the eluted volatiles. In conjunction with detection and evaluation of eluted volatiles through the olfactometer port, combination of mass spectrometry to the GC-O (GC-O-MS) will also permit the identification of the volatiles. CharmAnalysis™ is a GC-O dilution technique first developed to quantify the odour activity of individual components in natural products (Acree et al., 1984). The sensory attribute and duration of the perception of each odour compound exiting the GC as eluent are measured from start to end, and sniff runs are performed until dilutions of the injected sample, usually in factors of two, or three, present no more detectable odourous regions (Marin et al., 1988). The response of each compound over all the dilutions yield charm values, which are proportional to the amount of the stimulus and inversely proportional to the sniffer's threshold. With known amounts of compounds, detection thresholds of odourants may thus be obtained from GC-O dilution analysis.

1.5. Olfactory Sensitivity with Age

Nearly all the countries of the world are experiencing an ageing population due to decreasing mortality and declining fertility. The proportion of older people (aged 60 and above) has increased from 9.2 % to 11.7 % from

1990 to 2013 and is expected to grow to 21.2 % by the year 2050 (United Nations, 2013). While more people live longer lives, the prevalence of chronic diseases has also risen, impacting both social and economic systems. To achieve quality of life, healthy ageing, chronic disease management, proper nutrition and dietary habits are imperative.

Loss in olfactory function with age has been well-documented in in clinical reports, experimental and epidemiological studies (Doty, Shaman, & Applebaum, 1984a; Ship, Pearson, Cruise, Brant, & Metter, 1996; Schiffman, 1997). The causes of these age-related chemosensory losses are poorly understood and have been attributed to modifications in the olfactory epithelium from increased oxidative stress, decreased regeneration of receptor cells (Ahmed & Haboubi, 2010), cumulative damage to olfactory receptors and neurological degeneration (Wong, Muller, Kuwabara, Studenski, & Bohen, 2010; Doty, 2009).

The olfactory system is less robust than the taste system, and losses have been postulated to become more apparent after 60 years of age and increase in severity after 70 (Ship et al., 1996). As a consequence of age-associated olfactory loss, elevated odour thresholds and decreased cognitive identification of odours accompany the process of ageing, leading to lower perceived intensities and discrimination ability, and mitigates in odour perception (Cain, Reid, & Stevens, 1990; de Graaf, Polet, van Staveren, 1994; Kremer, Mojet, & Kroeze, 2007b).

Considerable variations exist in the degree of olfactory loss with increasing age due to confounding factors which include age-associated changes in cognitive function and memory, health status, education level, and

odour familiarity (Lehrner, Glück, & Laska, 1999; Zucco, Hummel, Tomaiuolo, & Stevenson, 2014). A growing number of studies have demonstrated heterogeneous loss in olfactory ability with age, showing that while some odourants and odour mixtures are sensitive to age effects, others seem to be unaffected (Wysocki & Gilbert, 1989; Konstantinidis, Hummel, & Larsson, 2006).

Notably, most evidence for loss of olfactory function with age are based on aggregate performance scores of a single olfactory measure or the combination of scores from various olfactory measures, and little is known about the influence of age on olfactory sensitivity towards specific single chemical compounds. Research performed to measure olfactory abilities have predominantly utilized odour mixtures for identification. However, odour mixtures can introduce suppression, enhancement, and adaptation interactions between the odourants in human perception (Frank, Goyert, & Hettinger, 2010; Grabenhorst, Rolls, Margot, da Silva, & Velazco, 2007; Shepherd, 2006).

When sensitivity towards one odourant, or odour type, is more affected by normal ageing than another, the perceived interaction between these two odours and the integrated perception of the final overall odour may be distorted, leading to failure in identification. Conversely, successful identification may also be aided by various odour cues in the mixture, even in the presence of loss in odourant olfactory sensitivity, herein the olfactory detection thresholds of the odourants. For instance, there are up to 400 volatiles identified in rose, but only a fraction of them is odour-active at the levels present, and a further fraction of those contribute to the distinctive aroma of rose at the ratios found naturally (Bianchi, Nuzzi, Leva, & Rizzolo,

2007). In spite of a loss in sensitivity towards a distinctive rose-like odourant in the mixture, the remaining odourants may be sufficient in driving towards successful identification. Thus, there is a need for an olfactory test to assess a subject's identification proficiency and threshold sensitivity for single odourants.

In addition, evidence is sparse concerning the relationship between olfactory measures, specifically identification and detection thresholds, of the same odourant or odour mixture. Unlike detection thresholds which require only low-level perceptual functions with low to no cognitive demands (Larsson, Finkel, & Pedersen, 2000), odour identification ability which is determined at suprathreshold is reliant on cognitive factors such as executive functioning, semantic memory, and episodic memory (Hedner, Larsson, Arnold, Zucco, & Hummel, 2010; Larsson et al., 2000; Larsson, Nilsson, Olofsson, & Nordin, 2004) to translate olfactory perception to lexicon (Olofsson et al., 2014). As a consequence, it comes as no surprise that while individuals who pay attention to olfactory information in everyday life excel in odour identification proficiency, such high olfactory awareness had no influence on threshold sensitivity (Arshamian, Willander, and Larsson, 2011). Although both identification ability and threshold sensitivity have been found to decrease with age, there may not be a necessary connection between the two olfactory measures. Accordingly, the olfactory test should be designed to utilize a single set of odourants for both tests of identification proficiency and threshold sensitivity in order to detect if a relationship exists between the two olfactory function measures.

1.6. Consequences of Loss in Olfactory Sensitivity with Age

Olfactory loss not only affects the quality of life and daily activities, such as the ability to detect gas leaks and spoilt foods, but also changes dietary behaviour (Miwa et al., 2001; Frasnelli & Hummel, 2005). The flavour of foods is perceived by all individuals. These flavour perceptions are formed by integrating our sensory modalities – predominantly smell, taste, and chemesthesis, and with influences of sight and sound, in the brain (Shepherd, 2006). While taste perception is limited to a few sub-modalities, i.e. sweet, salty, bitter, sour and umami, humans possess hundreds of olfactory receptors and can discriminate between more than a trillion odours (Bushdid et al., 2014), thus making the sense of smell crucial in food flavour perception. As flavour perception of food is an important aspect in food hedonics, cravings, and appetite (Shepherd, 2006; Yeomans, 2006), changes in olfactory sensitivity will have a negative impact on food intake and habits.

To meet the health needs of the ageing population, nutritious and balanced meals with a wide variety of foods are required to provide the right proportions of macro- and micro-nutrients for the body. An unintentional decline in food intake as a result of chemosensory losses can lead to malnutrition and possible reductions in body weight (Donini, Savina, & Cannella, 2003; Morley, 2001). The "anorexia of ageing", a physiological age-related reduction in appetite and energy intake leading to malnutrition and immune dysfunction, has been attributed to olfactory deficit (Hays & Roberts, 2006; Morley & Thomas, 1999). In contrast, decline in olfaction may also cause difficulty in adhering to a healthful diet due to lowered preference for sour fruits and bitter vegetables and increased intake of sweet, salty, and fatty

foods (Duffy, Backstrand, & Ferris, 1995; Mattes, 1990), which results in elevated risk of chronic diseases. Other nutrition-related diseases may include: cardiovascular diseases, diabetes, obesity, and hypertension (Rolls, 1999).

1.7. Compensation Techniques for Olfactory Sensitivity

In theory, intensification or enhancement of flavours will be able to compensate for the loss of chemosensory sensitivity of the elderly, and thus lead to stimulation of food intake in the elderly. However, strategies to compensate for changes in food palatability have yielded contradictory results. While some studies have chosen to increase the use of ingredients already present in the prepared foods for enhancement (Schiffman & Warwick, 1993), others added ingredients not previously found in the food (Kälviäinen, Roininen, Tuorila, 2003; Laureati et al., 2008). Moreover, the application of the enhanced flavours into food for different subject populations, such as the age ranges defined as "elderly", subjects' health states, or if subjects were institutionalized or free-living, limits the ability for comparison across studies. The complexity of food matrices, differing methods for compensation, and assumed uniform loss of olfactory sensitivity with age also affect the efficacy of compensation techniques (Griep, Mets, & Massart, 1997; Koskinen, Kälviäinen, & Tuorila, 2003a; Koskinen, Kälviäinen, & Tuorila, 2003b; Koskinen, Nemonen, & Tuorila, 2005; Laureati, Pagliarini, & Calcinoni, 2008).

As mentioned, ageing affects identification, discrimination, and detection of odour mixtures (Doty et al. 1984; Ship et al. 1996; Kobal et al.,

1996; Schiffman, 1997). Aromas we are exposed to in daily life consist of diverse chemicals of varied compositions and small changes in the ratios of these chemicals can result in considerable changes in odour perception. Little is known on differences in the extent of loss in sensitivity towards single odourants with normal ageing. In a food odour mixture, when sensitivity towards one odourant drops faster than another, the integrated perception of the food at different ages may be distorted. Thus, it is imperative to first gain insight on the extents of olfactory loss to specific single odourants for the elderly, in order to design targeted remedies for the effects of differential chemosensory losses.

Inconclusive and variable results from studies may be due to the fact that while losses in olfactory abilities with age have been well established, because of the diagnostic purpose of the research performed, most evidence available on ageing effects in relation to detection and identification of odourants were based on the use of a single compound, or aggregated performance scores from olfactory battery tests, with little or no emphasis on differences in the extents for loss of sensitivity towards specific odourants or odour types. Another issue with the use of a single compound for detection threshold testing is the variation in individual's threshold from time to time, and test to test (Stevens, Cain, & Burke, 1988).

Moreover, olfactory performance scores have been shown to have little to no correlation with perceived intensity of food items (Kremer, Bult, Mojet, & Kroeze, 2007a; Seo & Hummel, 2009), such that there is limited translation from scored olfactory performance to real life. Studies on olfactory loss have been largely based on two general populations of "the young" and "the elderly"

(Kälviäinen et al., 2003; Kremer et al. 2007a), with little information available on the changes in olfactory performance across the full lifespan.

In a three-week study, Schiffman and Warwick (1997) discovered that while increasing flavour intensity of various foods encouraged higher intake of enhanced foods for the elderly, overall food intake remained the same with decreased intake of other foods. Therefore, enhancement of flavours for select nutritious foods that are deemed unpleasant for the ageing population to entice and encourage intake by the elderly may be the solution to maintaining a long-term balanced and nutritional diet for the ageing population.

1.8. Flavour Modification

A flavour is composed of a mixture of volatiles which may belong to any number of odour types. For instance, in a natural mango fruit, odour-active volatiles may include floral (contributed by compounds such as phenylethyl alcohol, ethyl phenylacetate), fruity (ethyl butanoate, 2-methylpropanoate), green (hexanal, (E)-2-hexenal), and caramel (ethyl maltol, 2,5-dimethyl-4-methoxy-3(2H)-furanone) notes (Pino, Mesa, Muñoz, Marti, & Marbot, 2005). If olfactory sensitivity towards each odourant decreases at different rates with age, the resulting flavour perception of the mango may be distorted and unpleasant. As a result, instead of compensating for olfactory losses of the elderly, the flavour enhancement technique used in previous studies merely intensified the overall imbalanced flavour and failed to address the issue with heterogeneous olfactory sensitivity with age.

To address the differential rates of olfactory decline, the current study proposed a flavour modification method in place of the flavour enhancement method. Instead of the uniform increase of intensity for all the components in a flavour, the flavour would be modified by adjusting only concentrations of odourants or odour types affected by age. The extent of modification of each odourant or its odour type would depend on the degree of olfactory loss for the aforementioned odourant. By addressing individual components of a flavour, it was hoped that distorted flavour perception as a result of heterogeneous olfactory loss may be redressed, thereby increasing liking or enhanced palatability for the modified flavour.

In compensatory strategies adopted for the loss of olfactory sensitivity with age taken by other researchers, the use of complex food systems can also further complicate results. Apart from smell and taste, visual cues (Philipsen, Clydesdale, Griffin, & Stern, 1995; Seo & Hummel, 2009), textural cues (Kälviäinen et al., 2003; Kremer et al., 2005; Kremer et al. 2007b), as well as chemesthesis (Koskinen et al., 2003b), can all interact to give rise to an overall flavour perception. Furthermore, the varying rates of changes in sensitivities of the young and elderly to different sensory modalities affects food preferences as well (Kälviäinen et al., 2003; Kremer et al., 2007a; Kremer et al., 2007b; Zandstra & de Graaf, 1998). Flavour enhancement may hence be successfully implemented only for specific types of food systems.

To evaluate if flavour modification may improve palatability of foods for the elderly, the flavours have to be applied into a medium which minimises influence from other sensory modules, such as touch and sound. The use of beverages minimises the influence of tactile-flavour interactions which may

influence the overall flavour perception. Although taste-odourant interactions may vary for different concentrations of odourants applied into the beverages, the resulting changes in overall flavour perception can be attributed solely to the change in concentration of the odourants by keeping taste-contributing ingredients of the beverage applications constant.

1.9. Research Hypotheses

i. The loss of olfactory sensitivity with age is not uniform across odourants for an ethnic Chinese Singaporean population.

ii. The relationship between identification ability and odour detection threshold of single odourants remains constant with age.

iii. Modification of flavours to compensate for odourant-specific olfactory loss will increase liking for the flavours by elderly subjects.

1.10. Research Objectives and Outline

In order to elucidate the changes in olfactory functions with age and cater to the palatability of the elderly, much work needed to be done. To move through the development of an olfactory test to the modification of flavours for consumer evaluation, this study will address the following objectives:

- To design, develop and validate the Specific Sensitivity Test, a test for the measurement of odour identification ability and detection threshold sensitivity, for the population of Asians – specifically Singaporeans and Singapore PRs of Chinese ethnicity (Chapter 2),

- To evaluate and compare the threshold subtest of the Specific Sensitivity Test with GC-O dilution analysis (Chapter 3),
- To assess the olfactory function of Singaporeans and Singapore PRs of Chinese ethnicity, aged 21 to 80, using the Specific Sensitivity Test and examine changes in olfactory function for single odourants with age (Chapter 4),
- To elucidate the relationship between identification ability and detection threshold from results of the Specific Sensitivity Test (Chapter 5), and
- To utilize the flavour modification method in flavours for beverage applications and determine the ability of the technique to compensate for differentiated olfactory losses of the elderly (Chapter 6).

CHAPTER 2: DEVELOPMENT OF THE SPECIFIC SENSITIVITY TEST

2.1. Introduction

As the Sniffin' Sticks test was designed primarily for clinical usage to diagnose olfactory deficits, and to measure olfactory loss with disease states (Hummel et al., 1997), odourants not associated with food products were primarily used, such as petroleum oil and leather. The overarching aim of our research is to characterize the age-related loss of olfactory sensitivity to food-associated odours to inform flavour enhancement decisions for the elderly palette. Therefore, a quantitative olfactory performance test consisting only of food-associated odour-active compounds, or odourants, appropriate for the assessment of olfactory abilities of a Singapore population was designed and validated through preliminary trials using Sniffin' Sticks. Development of a quantitative olfactory performance test will enable its use in a larger population study on the changes in human olfactory sensitivity with age.

The measurement of loss of olfactory sensitivity to different food odours proposed in this research has not been performed elsewhere, thus there was no direct comparison of olfactory tests in literature to allow validation of method by parallel execution. Proposed validation for the Specific Sensitivity Test was done by comparison with results from Gas Chromatography-Olfactometry (GC-O) dilution analysis (Acree et al., 1984) (Chapter 3).

To develop an olfactory performance test assessing olfactory sensitivity for food odours and gain insight on the extents of olfactory loss to specific odourants or odour types for the elderly rather than mixtures, a quantitative olfactory performance test consisting only of food-associated odour-active compounds, or odourants, appropriate for the assessment of olfactory abilities of a Singapore population was designed and validated through preliminary trials using Sniffin' Sticks.

The Sniffin' Sticks were filled with *n*-butanol and new odourants, which have variable volatility, vapour pressures, solubility in propylene glycol, the solvent used as the carrier medium for the odourants. In addition to the chemical properties, differences in the source and purity of chemicals may also affect the reproducibility of the Specific Sensitivity Test. Thus, validation tests were conducted to ensure reproducibility and stability of the odourants in the filled Sniffin' Sticks. In addition, the Specific Sensitivity Test was performed with the Standard Sniffin' Sticks Identification and Threshold subtests in order to validate the technique utilized by the researchers.

The successful development of a quantitative olfactory performance test would enable its use in a population study to determine how human olfactory sensitivity change with age of the ethnic Chinese Singaporeans.

2.2. Aims & Objectives

This study first selected for individual single chemical odourants to be used in the Adapted Specific Sensitivity Test (ASST) based on a survey of 205 Singaporean and Singapore Permanent Residents (PRs). From the selection of odourants, a quantitative Adapted Specific Sensitivity consisting of food-associated odours appropriate for the assessment of age-related changes in olfactory capabilities – identification ability and odour threshold levels, for a healthy Singapore population, was designed and validated with 20 subjects of various adult age groups from 21 to 80 years old.

2.3. Materials and Methods

2.3.1. Preliminary Survey

Odours used in identification tests should be familiar to the test subjects. Hence, selection of odours familiar to the Singapore population was performed by surveying 205 Singaporeans and Singaporean Permanent Residents (age range: 18-80; 110 males, 93 females and 2 no responses) regarding their familiarity with 75 food-associated odours gathered from established olfactory tests (Cho et al., 2009; Doty, Sharman, & Dann, 1984a; Doty et al., 1996; Kobal et al., 2000) and additional odours commonly experienced in Singapore, such as durian, caramel and vanilla (Appendix 1). The surveyed population rated the familiarity of the odours on a numerical scale of 1 as “Not familiar at all” and 5 as “Know the smell very well”. Selection of odours to evaluate olfactory sensitivity was determined by high familiarity scores, and availability of single chemicals that are able to

correspond with the odour. Considerations were also made to exclude odours that are familiar only to specific age groups by averaging mean scores from every age group, such as chocolate, pizza, and lime, which were less familiar to the older age groups, and milk and sesame oil, which were less familiar to the younger age groups.

2.3.2. Volunteers Recruitment and Demographic Information

Twenty healthy subjects, split into decade-long age groups (21-30: 4, 31-40: 4, 41-50: 4, 51-60: 3, 61-70: 4 and 71-80: 1) were recruited for the preliminary ASST and validation tests to determine olfactory sensitivity in the individuals.

Demographic data was obtained from subjects in the form of a questionnaire before conducting the ASST. Information obtained include age, gender, race, pregnancy status, smoking status, description of work environment (home or unemployed, factory, office, outdoors, others), perception of own sense of smell, usage frequency of perfume, cologne or after-shave, and presence of diagnosed illnesses and allergies.

2.3.3. Preliminary Adapted Specific Sensitivity Test

2.3.3.1. Sniffin' Sticks Preparation

Ten odour-active chemicals (odourants) were selected to represent ten familiar odours of different odour types from Preliminary Survey findings, and were used for identification and threshold tests (Table 1). The ten odourants were 1-pyrazin-2-ylethanone (≥ 99 %, Frutarom, UK), butanoic acid

(Frutarom, UK), decanal (≥ 98 %, Berjé Inc., USA), 3-methylbut-1-yl ethanoate (≥ 99 %, Frutarom, UK), 2-methyloxolane-3-thiol (≥ 98 %, R.C. Treatt & Co, UK), 2-phenylethanol (≥ 95 %) and (2*E*)-3-phenylprop-2-enal (≥ 98 %) (Citrus & Allied Essences, USA), and 2,6-dimethoxy-4-methylphenol (≥ 98 %), (2*S*,5*R*)-2-isopropyl-5-methylcyclohexanon (≥ 96 %), and oct-1-en-3-ol (≥ 98 %) (Sigma-Aldrich, USA). Carrier medium for odourants was propylene glycol (≥ 99.5 % weight, Dow Chemical, USA), and *n*-butanol (≥ 99.9 %, Sigma-Aldrich, USA) was used in the adapted Specific Sensitivity Test. The odourants used were all food grade and comply with the Food Chemicals Codex (FCC) standards.

Odourants were presented in felt-tip pens, also known as Sniffin' Sticks (Burghart Instruments, Wedel, Germany), which are approximately 14 cm long and has an inner diameter of 1.3 cm. These pens were filled with odourants dissolved in propylene glycol instead of liquid dye. For the threshold tests, empty Sniffin' Sticks were each filled with 4 mL of 12 dilutions of each odourant, prepared in geometric series starting from the highest concentration (Table 1) (dilution ratio 1:2 in propylene glycol). Sniffin' Sticks used for the Adapted Specific Sensitivity Identification Test (ASSIT) were selected based on the dilutions of odourants which best reflect the desired descriptor.

For all odour presentations, the experimenter removed the cap for approximately 3 seconds, and the pen's tip was placed approximately 2 cm in front of both nostrils of the subject. All tests were performed in a quiet, well-ventilated room.

Table 1. Odourants and concentrations of odourants used in the Preliminary Adapted Specific Sensitivity Test.

Odourant Name (IUPAC)	Odourant Name (Common)	Adapted Identification Test Concentration [% (v/v)]	Highest Threshold Test Concentration for Adapted Threshold Tests [% (v/v)]
Butan-2-ol	*n*-Butanol	-	36.0
Decanal	Decanal	0.54	0.54
3-Methylbut-1-yl ethanoate	Isoamyl acetate	0.60	1.80
2-Phenylethanol	Phenylethyl alcohol	0.30	0.30
(2*E*)-3-Phenylprop-2-enal	Cinnamaldehyde	0.405	0.405
1-Pyrazin-2-ylethanone	Acetyl pyrazine	0.162	0.162
(2*S*,5*R*)-2-Isopropyl-5-methylcyclo-hexanone	Menthone	3.20	28.8
Oct-1-en-3-ol	1-Octen-3-ol	0.36	0.36
2,6-Dimethoxy-4-methylphenol	4-Methyl-2,6-dimethoxyphenol	0.54	0.54
Butanoic acid	Butyric acid	2.70	2.70
2-Methyloxolane-3-thiol	2-Methyl-3-tetrahydrofuran thiol	0.0038	0.0342

2.3.3.2. Adapted Specific Sensitivity Identification Test (ASSIT)

Ability to identify odours was assessed by presenting the subjects with the list of selected odourants filled in Sniffin' Sticks. As in the Standard Sniffin' Sticks Identification test (SSSIT), the Adapted Specific Sensitivity Identification Test (ASSIT) used a multiple forced-choice design. Panelists were asked to identify the best label for the identity of the odour presented to

them from a list of 4 descriptors (Table 2). Panelists were permitted to re-sample the odours as many times necessary to make a decision, but an interval of 30 seconds was kept between sniffs to prevent olfactory desensitization.

Apart from familiarity with the odourants, odourants used need to be balanced in intensity to ensure homogeneity of the test. Although the selected odourants were roughly matched for odour intensity prior to testing, in the preliminary trials, panelists were also asked to rate the intensity of the odourants on visual rating scales from 0 to 5 after identification of the odourant. Intensity was rated 0 if there was "No Smell Detected" and 5 if "Very Strong".

Table 2. Identifying descriptors and distractors for each odourant of the Adapted Specific Sensitivity Identification Test.

Odourant	Odour Type	Identifying Odour Descriptor	Distractors		
			#1	#2	#3
Decanal	Citrus	Orange	Peppermint	Smoke	Apple
Phenylethyl alcohol	Floral	Rose	Mint	Strawberry	Peach
Isoamyl acetate	Nutty	Popcorn	Strawberry	Coconut	Banana
Acetyl pyrazine	Fruity	Banana	Walnut	Cherry	Peach
Cinnamal-dehyde	Spicy	Cinnamon	Vanilla	Chocolate	Honey
L-Menthone	Minty	Mint	Rose	Carrot	Garlic
4-Methyl-2,6-dimethoxy-phenol	Smoky	Smoke	Salami	Grass	Leather
Butyric acid	Dairy/ Rancid	Cheese	Fish	Bread	Ham
2-Methyl-3-tetrahydro furanthiol	Alliaceous	Onion	Almond	Cheese	Peppermint
1-Octen-3-ol	Earthy	Mushroom	Mustard	Clove	Wood

2.3.3.3. Adapted 10-Item Threshold Test & Adapted Specific Sensitivity Threshold Test

Olfactory threshold determination using the Adapted 10-Item Threshold Test (ATT10) for the 10 selected chemicals and Adapted Specific Sensitivity Threshold Test (ASSTT) for *n*-butanol, which is used in the Standard Sniffin' Sticks Threshold subtest (SSSTT), were run similarly to SSSTT for olfactory threshold determination. Subjects were blindfolded and assessed for olfactory sensitivity towards odourants using a single-staircase, 3-alternative forced-choice procedure (Haehner et al., 2009). Three pens were presented in random order, one containing a certain odourant dilution, the other two containing only the solvent, so that the subject was tasked to identify the pen with the odourant. Threshold determination started at the highest dilution (e.g. 12), working down (e.g. at intervals of 4). Upon two consecutive identification of the pen with the odourant at a certain dilution (e.g. 8), the next higher dilution step is offered to the subject (e.g. 9). Until the next turning point when the odourant is not identified, then a lower dilution was offered until 7 turning points are established. The threshold was defined as the mean of the last 4 staircase reversals. The order of the sets of odourants was randomised for each subject.

2.3.4. Method Validation Tests

2.3.4.1. Standard Sniffin' Sticks Tests

The adapted identification and threshold tests were compared with the original 16-items identification and threshold subtests, Standard Sniffin'

Sticks Identification Test (SSSIT) and SSSTT respectively, from the Standard Sniffin' Sticks battery test (Burghart Instruments, Wedel, Germany).

The SSSTT relied on a single odourant, *n*-butanol (highest concentration at 4 % in propylene glycol) and consists of 16 dilution levels (Kobal et al., 1996). As *n*-butanol has a neutral odour, and has low variability in odour detection threshold across subjects (Doty, McKeown, Lee, & Shaman, 1995; Hummel et al., 1997; Stevens et al., 1988), it is a widely popular choice in the determination of odour detection threshold in olfactory performance tests. To ensure that the preparation technique of this study did not result in differing results from the standard threshold subtest, *n*-butanol threshold determination was included in adapted threshold tests (Section 3.3.3). However, as the SSSTT dilutions were perceived by the researchers to have higher odour intensities at the same dilutions, thus the highest concentration for the Adapted Specific Sensitivity Threshold test (ASSTT) was adjusted to 36 % (v/v) while keeping the dilution ratio constant. The ASSTT also consists of 12 dilution levels, in line with the rest of the odourants in ATT10 (Table 1).

2.3.4.2. Test-Retest Reliability

In order to verify the internal consistency and stability of the preliminary Adapted Specific Sensitivity Test, all standard and adapted tests were performed again in the 20 subjects after an interval of 32 to 109 days (mean ± standard deviation$_{20}$ = 67 ± 25). The test procedures were kept consistent in the two sessions as described in section 2.3.3. In both sessions, the Standard Sniffin' Sticks tests (Section 2.3.4.1) were conducted first before

the Adapted Specific Sensitivity tests. Feedback was not given to participants after the completion of the first session.

2.3.5. Statistical Analysis

Statistical analyses were performed by SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA) for Macintosh. To explore identification and threshold scores in relation to age and gender, data was analysed with one-way analysis of variance (ANOVA) and post-hoc Tukey's test. Pearson's correlation analyses were used to examine relationships between standard and adapted tests, and test-retest reliability of the Adapted Specific Sensitivity Test. Partial correlation analyses were then performed to control for effects by various factors on test-retest reliability. Student t-test was used to compare results from test-retest sessions. Alpha level was set at 0.05 for all tests. Unless otherwise stated, mean values are expressed as mean ± standard error of the mean (SEM).

2.4. Results

2.4.1. Preliminary Survey Results

About half the surveyed population was aged 21-30 (48.5%), and the least surveyed group was those of ages 71-80 (1.5%). Of the 75 food odours surveyed, the surveyed population as a whole rated durian, coffee, garlic, onion, and orange to be the most familiar odours (odour familiarity $\geq$ 95%), while the most familiar aromas across all age groups differed slightly: durian, garlic, coffee, fish, lemon, and orange (odour familiarity by age $\geq$ 4.27).

In spite of high familiarity scores for some odours, the list of food odours for identification and threshold determination tests (Table 3) were selected because they were all naturally occurring food constituents, had high familiarity ratings, covered a range of odour types, and had single chemical compounds that reflect the desired odour descriptors corresponding to these odour types.

Table 3. Descriptive statistics of odourants in the Adapted Specific Sensitivity Identification Test.

Odourant Descriptor	Chemical Odourant	Odour Type	Odour Familiarity[1] (%)	Mean Odour Familiarity[2] (Rating)	Odour Familiarity by Age[3] (Rating)
Orange	Decanal	Citrus	95	4.25	4.27
Banana	Isoamyl acetate	Fruity	90	4.13	4.17
Rose	Phenylethyl alcohol	Floral	78	3.56	3.65
Cinnamon	Cinnamal-dehyde	Spicy	83	3.82	3.70
Popcorn	Acetyl Pyrazine	Nutty	88	3.98	4.01
Mint	Menthone	Minty	87	4.12	4.18
Mushroom	1-Octen-3-ol	Earthy	82	3.60	3.58
Smoke	3-Methyl-2,6-dimethoxy-phenol	Smoky	95	4.29	4.11
Cheese	Butyric acid	Rancid	76	3.43	3.35
Onion	2-Methyl-3-tetrahydro-furan thiol	Savoury	95	4.22	4.07

Familiarity was rated on a 5-point numerical scale from 1 = "Not familiar at all" to 5 = "Know the smell very well".

[1] Odour Familiarity (%) indicates the percentage of subjects who rated the odour with a score of ≥ 3;

[2] Mean Odour Familiarity (Rating) indicates the mean rating of the odour ($N = 205$) by all subjects;

[3] Odour Familiarity by Age (Rating) indicates the mean of mean ratings of the odour taken from each age group [21-30 ($n = 4$), 31-40 ($n = 4$), 41-50 ($n = 4$), 51-60 ($n = 3$), 61-70 ($n = 4$), 71-80 ($n = 1$)].

2.4.2. Preliminary Adapted Specific Sensitivity Test

2.4.2.1. Method Validation

To compare between the SSSIT and ASSIT, ASSIT identification scores of each subject were plotted against corresponding SSSIT Identification Scores (Figure 1). SSSIT and ASSIT scores were calculated as the total number of correct odour identification out of the 16- and 10-item tests, respectively. Coefficients of correlation between the identification tests, SSSIT and ASSIT, were low and not statistically significant for both the initial test (r_{20} = -0.07, p = 0.78) and retest sessions (r_{20} = 0.40, p = 0.08).

In addition, mean identification rates (±SEM) using the SSSIT [$(0.81 \pm 0.02)_1$, $(0.80 \pm 0.02)_2$] were significantly higher than those for ASSIT [$(0.67 \pm 0.04)_1$, $(0.67 \pm 0.04)_2$] [t_1 (19) = 3.45, p_1 = 0.003, t_2 = 3.95, p_2 = 0.001]. Identification rates below 0.70 were observed in SSSIT for odours with descriptors: “leather” (0.55, 0.50), “turpentine” (0.45, 0.45) and “apple” (0.50, 0.35), while in the ASSIT, odourants with descriptors: “cinnamon” (0.55, 0.65), “orange” (0.50, 0.35), “popcorn” (0.50, 0.32), and “smoke” (0.35, 0.25).

To compare between the *n*-butanol detection thresholds of subjects using the SSSTT and ASSTT, ASSTT score of each subject was plotted against the SSSTT score for the first and second sessions (Figure 2). The higher the threshold score, the higher the olfactory sensitivity of the subject towards the odourant, as the threshold score indicates the highest dilution perceived by the subject. The score of SSSTT was calculated against a maximum of 16 while that of ASSTT was against a maximum of 12. In contrast to the identification tests, the coefficients of correlation between

threshold scores of SSSTT and ASSTT determined in the first and second sessions (Figure 2) were relatively high and statistically significant, at r_{20} = 0.54 (p = 0.02) and r_{20} = 0.68 (p = 0.001), respectively.

While the standard and adapted identification tests, SSSIT and ASSIT, were not comparable, the standard and adapted threshold tests, SSSTT and ASSTT, were closely related.

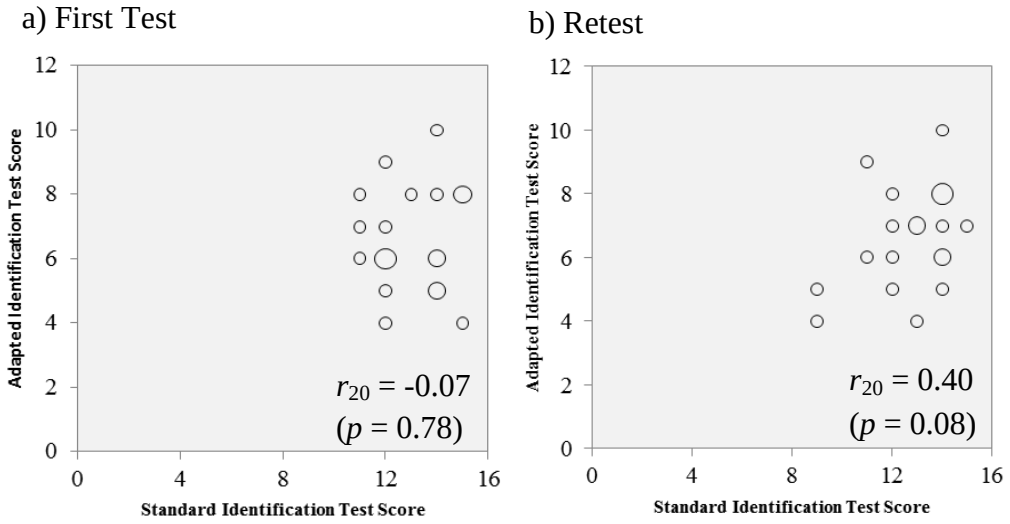


Figure 1. Correlations between Standard Sniffin' Sticks and Adapted Specific Sensitivity Identification Tests (N = 20) from two test sessions; a) First test, b) Retest. The largest circle shows three data points, next largest two, and the smallest one shows one datum. Coefficients of correlation and significance of correlation are indicated on the chart.

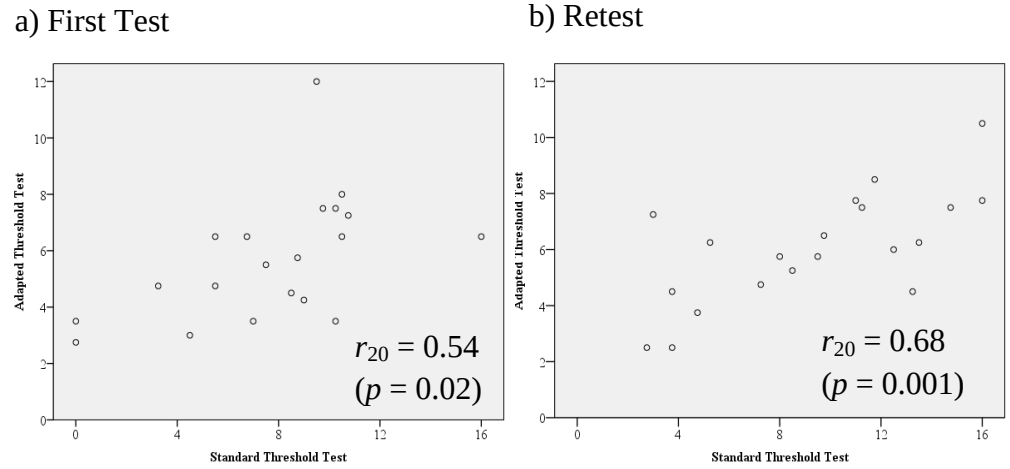


Figure 2. Correlations between Standard Sniffin' Sticks and Adapted Specific Sensitivity Threshold Tests (N = 20) using *n*-butanol from two test sessions; a) First test, b) Retest. Each circle represents one data point. Coefficients of correlation and significance of correlation are indicated on the chart.

2.4.2.2. Test-Retest Reliability

2.4.2.2.1. Standard Sniffin' Sticks and Adapted Specific Sensitivity Identification Tests

To evaluate the reproducibility of SSSIT and ASSIT, Pearson's correlation was obtained between identification test scores of the two test sessions (Figure 3). The Pearson's coefficient of correlation for test-retest sessions of the ASSIT ($r_{20} = 0.69$, $p = 0.001$) was statistically significant, and higher than that of the SSSIT ($r_{20} = 0.17$, $p = 0.47$). However, like the ASSIT [$(6.7 \pm 0.4)_1$, $(6.7 \pm 0.4)_2$] [t (19) = 0, $p = 1.0$], there was no significant difference between the mean identification scores of the initial (12.9 ± 0.3) and retest (12.8 ± 0.4) sessions for SSSIT [t (19) = 0.22, $p = 0.83$].

To evaluate the reproducibility of SSSTT and ASSTT, Pearson's correlation was also obtained between threshold test scores of the two test sessions (Figure 4). The SSSTT showed relatively good test-retest reliability ($r_{20} = 0.54$, $p = 0.01$), and there was no significant difference in mean threshold scores between the initial (7.65 ± 0.85) and retest (9.31 ± 0.97) sessions [t (19) = -1.89, $p = 0.08$]. On the other hand, test-retest reliability for the ASSTT was not statistically significant ($r_{20} = 0.36$, $p = 0.12$), but there was no significant difference in mean threshold scores of the first (5.64 ± 0.49) and retest (6.04 ± 0.49) sessions [t (19) = -0.75, $p = 0.75$].

a) Standard Sniffin' Sticks Identification Test

b) Adapted Sniffin' Sticks Identification Test

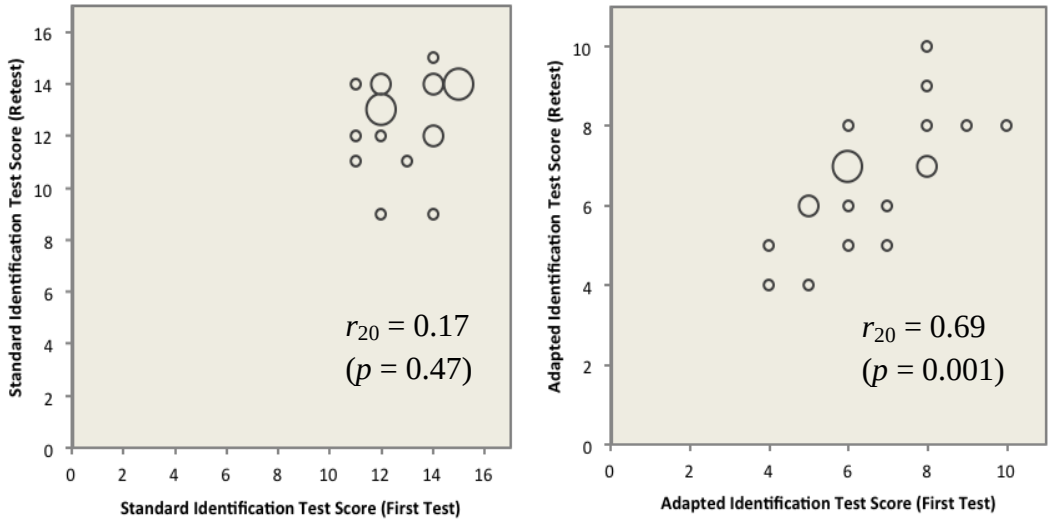


Figure 3. Correlation between test-retest of a) Standard Identification Test and b) Adapted Identification Test (N = 20). The larger the circle, the more data points converge on that coordinate. The largest circle shows three data points, next largest two, and the smallest one shows one datum. Coefficients of correlation are indicated on the chart.

a) Standard Sniffin' Sticks Threshold Test

b) Adapted Sniffin' Sticks Threshold Test

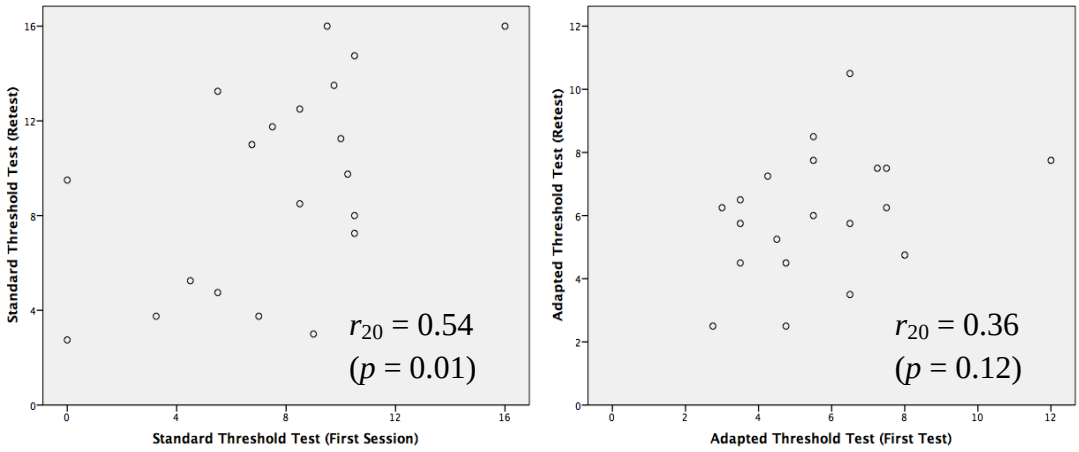


Figure 4. Correlation between test-retest sessions of a) Standard Sniffin' Sticks Threshold Test and b) Adapted Sniffin' Sticks Threshold Test (N = 20) using *n*-butanol. Coefficients of correlation are indicated on the chart.

2.4.2.2.2. Adapted 10-Item Threshold Test

Composite mean threshold score of the 10 odourants in the ASSTT was relatively high and statistically significant [$r_{20} = 0.57$, $p = 0.01$)]. However, results for individual tests for Pearson's correlation between the two sessions for each odourant in the standard and adapted tests were dependent on a few factors. Test-retest for SSSTT [$(7.65 \pm 0.85)_1$, $(9.31 \pm 0.98)_2$], orange, rose and cinnamon had statistically significant coefficients of correlation, while the rest of the odourants had low to no correlation between the threshold scores (Table 4).

Partial correlation analyses between the first test and retest were performed, controlling for the effects of the following factors: "number of days apart", "difference in time of day", "difference in last meal time", and "age". When controlling for "number of days apart" and "difference in time of day", banana and mint showed higher and statistically significant coefficients of correlation, and controlling for "difference in last meal time" had a positive effect on the coefficient of correlation for ASSTT and popcorn, indicating the influence of these factors on the reliability of test sessions of ASSTT and ATT10. The factor "age" did not affect test-retest reliability of the odourants.

While there was no statistically significant correlation for mushroom, smoke and onion, paired t-test showed no significant difference in mean threshold scores of the test sessions for these odourants. Among all the odourants in the ATT10, only cheese showed both low correlation, regardless of factors, and significant difference in mean threshold scores between tests sessions.

Table 4. Coefficients of correlation for test-retest of Standard Sniffin' Sticks and Adapted Specific Sensitivity for 10+1 Threshold Tests.

Odourant	Correlation r_{20}	Correlation, r_{16} Controlling for "Days"[1] and "Time"[2]	Correlation, r_{17} Controlling for "Last Meal"[3]	Paired t-test, t[4] df = 19
n-Butanol (SSSTT)	0.54*	0.53*	0.54*	-1.89
n-Butanol (ASSTT)	0.36	0.40	0.48*	-0.75
Orange	0.75*	0.83*	0.75*	-4.09*
Banana	0.34	0.57*	0.43	-1.64
Rose	0.54*	0.62*	0.55*	-1.48
Cinnamon	0.44*	0.49*	0.41	-1.88
Mushroom	0.22	0.20	0.20	-1.67
Popcorn	0.38	0.46	0.55*	2.04
Mint	0.42	0.48*	0.42	-1.44
Smoke	0.29	0.35	0.34	-1.58
Cheese	0.02	0.26	-0.03	-2.19†
Onion	0.43	0.43	0.37	1.28

* Correlation is significant at $p \leq 0.05$.

† The t-value is significant at $p \leq 0.05$.

[1] Number of days between the first test session and the retest.

[2] Difference in the time of day (Morning/Afternoon) between the first test session and the retest.

[3] Difference in the number of hours food was consumed before the first test session and retest.

2.4.2.3. Evaluation of Odourants in the Adapted Specific Sensitivity Identification and Threshold Tests

As the Preliminary Survey did not involve direct sampling, a test of olfactory identifiability and intensity ratings served to ascertain the rightful usage of chosen odourants for the Specific Sensitivity Test, and the appropriateness of levels used.

The mean intensity ratings (± 25%) of all odourants for test and retest sessions were 3.28 (± 0.82) and 3.34 (± 0.83), respectively. Although a significant increase in the mean intensity rating was found for smoke in the retest session from the first test (Table 5), no significant difference was observed in smoke mean threshold scores [t (19) = -1.58, p = 0.13]. Odours that are difficult to identify tend to give lower relative intensity ratings in spite of the true odour intensity (Doty, Shaman, & Applebaum, 1984b). Since there was no significant difference in popcorn mean threshold scores between the two test sessions [t (19) = 2.04, p = 0.06], the fall in identification rate for popcorn in the second test session was likely to have caused the drop in mean intensity rating.

The overall mean threshold scores for the first (5.08 ± 0.17) and second (5.68 ± 0.17) sessions were significantly different [t (199) = -3.681, p < 0.001] (Table 5), indicating an increase in subjects' performance in threshold in the retest. Specifically, there was a significant increase in mean threshold scores for cheese and orange, but no correlation was found between threshold scores and the number of days from Sniffin' Sticks preparation for both (cheese: r = -0.24, p = 0.14; orange: r = 0.20, p = 0.90).

Table 5. Results of 10 odourants in the Preliminary Adapted Specific Sensitivity Test.

Odourant	Mean Identification Rate[1]		Intensity Rating Mean (± SEM)		Mean Threshold Scores Mean (± SEM)	
	First Test	Retest	First Test	Retest	First Test	Retest
Banana	0.90	1.00	3.80 ± 0.23	3.65 ± 0.17	4.52 ± 0.44	5.54 ± 0.61
Mint	0.90	0.95	4.15 ± 0.17	4.10 ± 0.14	7.22 ± 0.40	7.78 ± 0.30
Onion	0.90	0.90	3.65 ± 0.22	3.95 ± 0.20	6.10 ± 0.12	5.91 ± 0.15
Cheese	0.75	0.80	3.75 ± 0.20	3.80 ± 0.23	6.71 ± 0.51	8.15 ± 0.42*
Mushroom	0.65	0.70	3.40 ± 0.32	3.50 ± 0.20	4.45 ± 0.37	5.28 ± 0.41
Rose	0.60	0.75	2.70 ± 0.31	2.80 ± 0.33	4.39 ± 0.69	5.38 ± 0.71
Cinnamon	0.55	0.65	2.95 ± 0.27	2.90 ± 0.26	3.41 ± 0.42	4.16 ± 0.32
Orange	0.50	0.35	2.85 ± 0.26	3.10 ± 0.19	4.43 ± 0.36	5.69 ± 0.47*
Popcorn	0.50	0.30	3.80 ± 0.16	3.05 ± 0.28*	6.59 ± 0.46	5.29 ± 0.65
Smoke	0.35	0.25	1.70 ± 0.25	2.50 ± 0.21*	2.96 ± 0.32	3.69 ± 0.43
All	0.66	0.67	3.28 ± 1.27	3.34 ± 1.11	5.08 ± 0.17	5.68 ± 0.17

* Retest mean rating was significantly different from the first test ($p < 0.05$).
[1] Mean Identification rate indicates the mean proportion of subjects who correctly identified the odourant.

2.4.3. Findings from the Preliminary Adapted Specific Sensitivity Test

In the first session, the full test required 4.5 hours of the subjects' time due to the inclusion of GC-O dilution analysis (Chapter 3), while the retest session took about 1.25 hours. Subjects were free to take short rest breaks between tests and odour threshold sets.

2.4.3.1. Relationship between Identification Ability and Threshold Sensitivity

To investigate the relationship between identification ability and threshold sensitivity, mean threshold scores of each odourant were calculated for individuals who correctly identified the odourant, i.e., correct identification, and those who identified the odourant as one of the other three distractors, i.e., incorrect identification. The threshold scores were then compared to determine if the ability to identify an odourant affected threshold sensitivity for it (Figure 5).

Of the odourants in ATT10, only cinnamon showed a significant difference ($p = 0.02$) in threshold scores between subjects who correctly identified the odour and those who did not. However, in the second session, the mean threshold score for cinnamon for subjects who identified the odourant successfully (4.13 ± 0.37, $n = 13$) was not significantly different from subjects who did not (4.21 ± 0.23, $n = 7$) ($p = 0.91$). In addition, there was no clear relationship between success in identification and mean threshold scores for the rest of the odourants, indicating that for the sampled population,

the ability to identify the preliminary Adapted Specific Sensitivity Test odourants did not have a significant impact on the items' threshold levels.

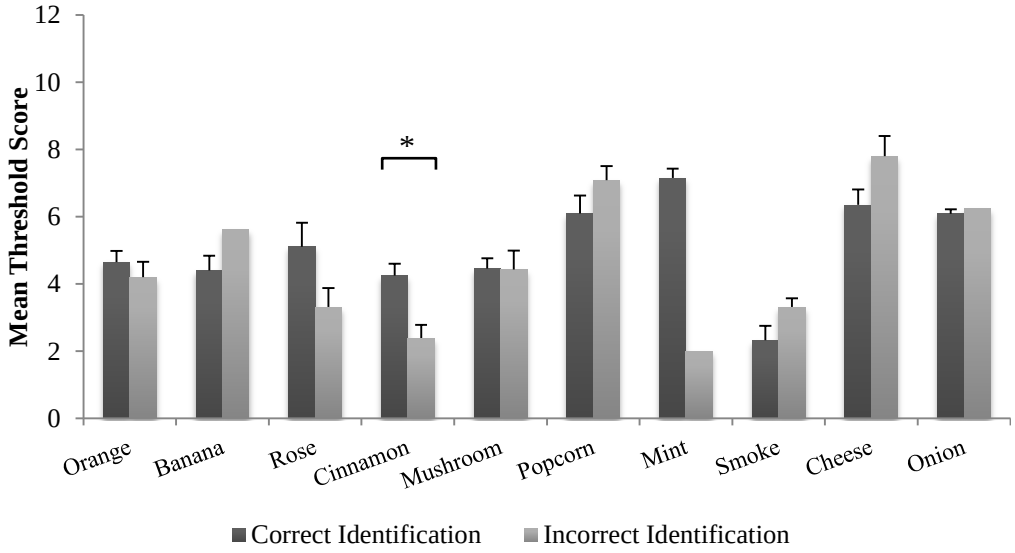


Figure 5. Relationship between correct and incorrect identification and mean threshold score (±SEM) of the preliminary Adapted Specific Sensitivity Test for each odourant. No error bars are shown for $n \leq 2$.
* Denotes significant difference in mean threshold scores ($p < 0.05$) of the odourant between subjects who correctly and subjects who incorrectly identified the odour.

2.4.3.2. Age Effects on Identification and n-Butanol Threshold

Grouping the subjects by decades, SSSIT mean identification rates did not reach significant difference between the age groups [F (5,14) = 0.09, p = 0.93], and mean identification rates for SSSIT remained constant with age (r_{20} = -0.004, p = 0.99). As can be observed from Figure 6, ASSIT identification rates fell significantly with age [F (5,14) = 2.94, p = 0.05]. However, when the 71-80 age group was excluded for post-hoc analysis because there was only one subject in that group, no significant difference was found between age groups with Tukey's test. Nevertheless, the fall in mean identification rate

with age for ASSIT was supported by a strong and statistically significant coefficient of correlation, r_{20} = -0.62 (p = 0.004), between age and ASSIT scores.

The decreasing trend was also observed in the findings of this study (Figure 7) for both the SSSTT and ASSTT, and the drop in threshold scores for both threshold tests were supported by significant coefficients of correlation between age and threshold scores, r_{20} = -0.49 (p = 0.03) for SSSTT and r_{20} = -0.57 (p = 0.01) for ASSTT. However, no significant differences were found between the age groups via ANOVA [SSSTT: F (5,14) = 2.77, p = 0.61; ASSTT: F (5,14) = 2.37, p = 0.09].

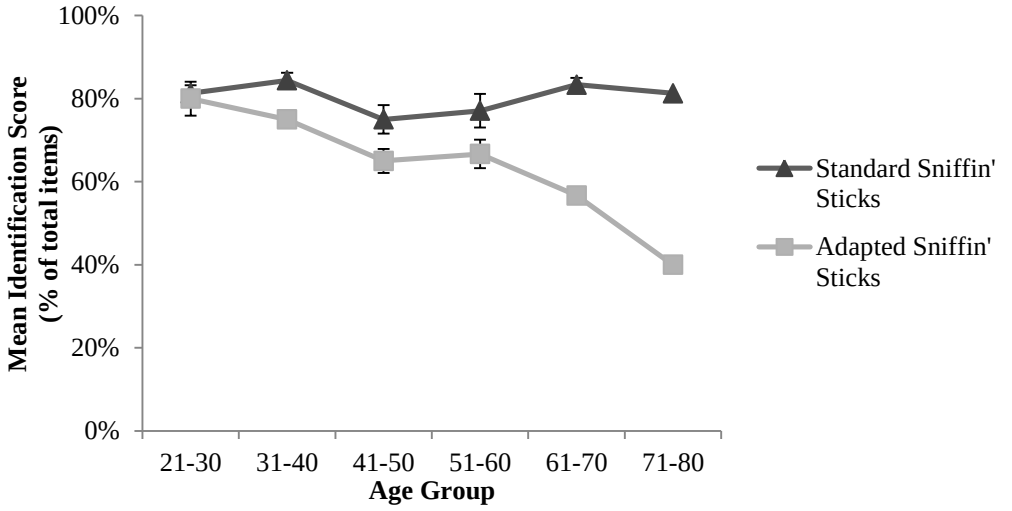


Figure 6. Age-related changes in mean odour identification rates (±SEM) from Standard Sniffin' Sticks and Adapted Specific Sensitivity Identification Tests in healthy subjects (n = 20) comprising of age groups: 21-30 (n = 4), 31-40 (n = 4), 41-50 (n = 4), 51-60 (n = 3), 61-70 (n = 4), 71-80 (n = 1) in the first test session. No error bars are given for $n \leq 2$.

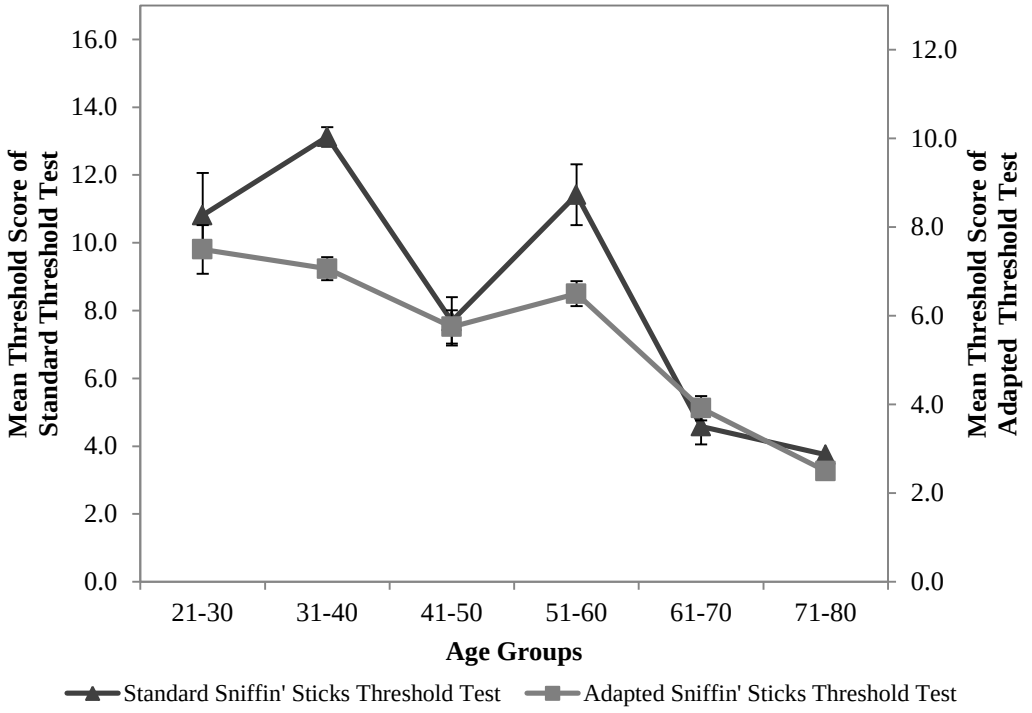


Figure 7. Age-related changes in threshold scores of *n*-butanol in Standard Sniffin' Sticks and Adapted Specific Sensitivity Threshold Tests (mean ± SEM) in healthy subjects (n = 20) comprising of age groups: 21-30 (n = 4), 31-40 (n = 4), 41-50 (n = 4), 51-60 (n = 3), 61-70 (n = 4), 71-80 (n = 1) in the first test session. No error bars are shown for $n \leq 2$.

2.4.3.3. Age and Threshold Sensitivity

In preliminary test findings for ATT10, apart from cinnamon [F (5,14) = 3.23, p = 0.04], no significant differences were observed in threshold scores between age groups by ANOVA for all the odourants (Figure 8) [orange: F (5,14) = 2.83, p = 0.06; Banana: F (5,14) = 0.93, p = 0.49; Rose: F (5,14) = 1.62, p = 0.22; Mushroom: F (5,14) = 0.24, p = 0.94; Popcorn: F (5,14) = 0.51, p = 0.76; Mint: F (5,14) = 0.31, p = 0.90; Smoke: F (5,14) = 2.07, p = 0.13; Cheese: F (5,14) = 1.74, p = 0.19; Onion: F (5,14) = 1.03, p = 0.44]. However, when the single 71-80 years old subject was excluded, no significant difference was found between the age groups with Tukey's post-hoc analysis.

Coefficient of correlation was only statistically significant for smoke with age (r_{20} = -0.60, p = 0.005) [orange: r_{19} = -0.18, p = 0.45; Banana: r_{19} = -0.29, p = 0.21; Rose: r_{19} = -0.21, p = 0.36; Cinnamon: r_{19} = -0.01, p = 0.98; Mushroom: r_{19} = -0.03, p = 0.90; Popcorn: r_{19} = -0.07, p = 0.78; Mint: r_{19} = -0.20, p = 0.39; Cheese: r_{19} = 0.38, p = 0.10; Onion: r_{19} = 0.10, p = 0.68]. Nevertheless, taking into account that there was only one subject in the age group 71-80, decreases in threshold scores with age can be observed for odourants orange, banana, rose, popcorn, mint and smoke, while cinnamon, mushroom, and onion were constant across the age groups, and in the case of cheese, increased with age.

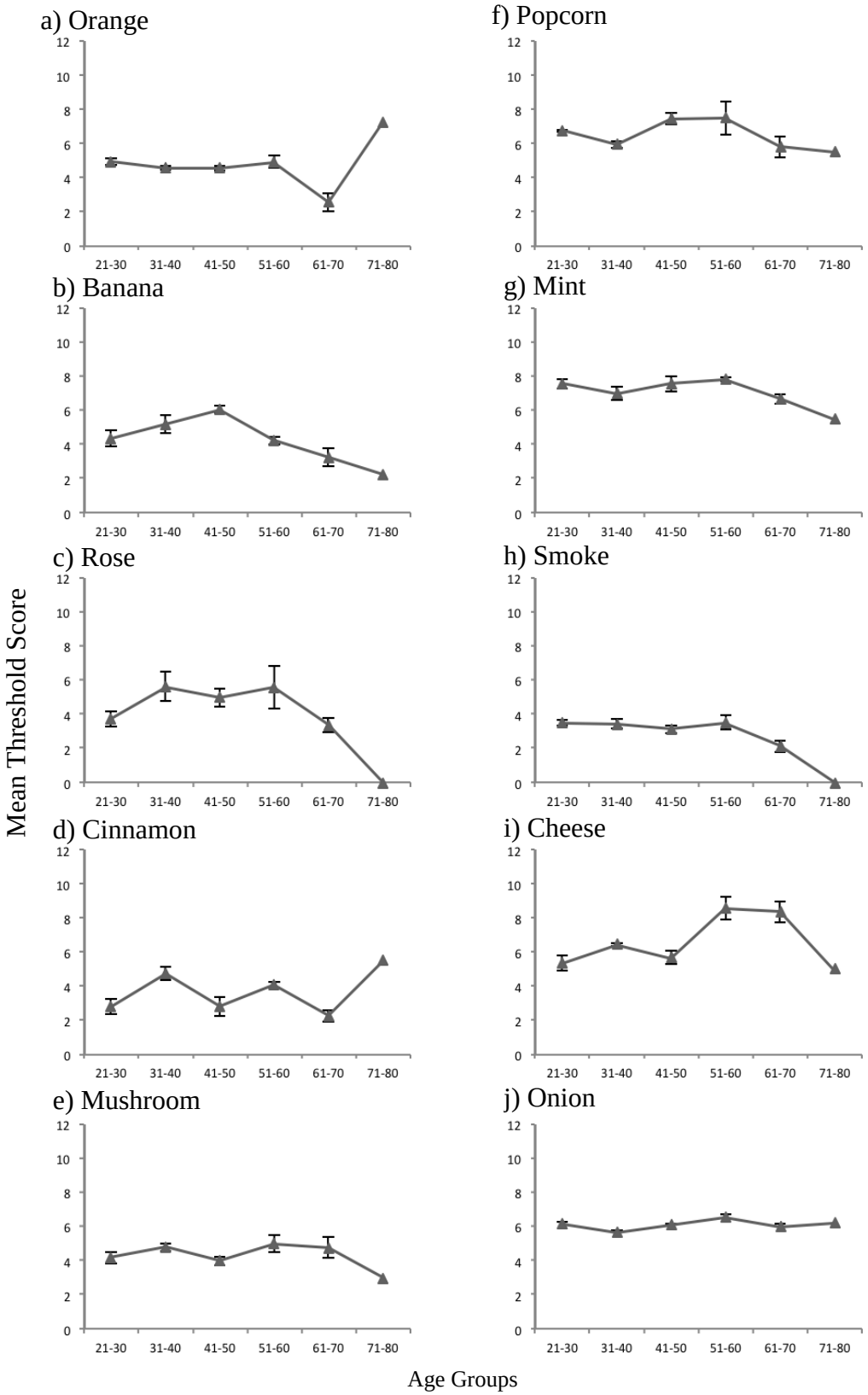


Figure 8. Age-related changes in mean threshold scores (±SEM) in each age group ($n_{21\text{-}30}$ = 4, $n_{31\text{-}40}$ = 4, $n_{41\text{-}50}$ = 4, $n_{51\text{-}60}$ = 3, $n_{61\text{-}70}$ = 4, $n_{71\text{-}80}$ = 1) determined from Adapted Threshold Test for odourants: a) Orange; b) Banana; c) Rose; d) Cinnamon; e) Mushroom; f) Popcorn; g) Mint; h) Smoke; i) Cheese and j) Onion. No error bars are shown for $n \leq 2$.

2.5. Discussion

2.5.1. Evaluation of the Adapted Specific Sensitivity Test

2.5.1.1. Identification Tests

The SSSIT and ASSIT were not comparable identification tests. Although the Adapted Specific Sensitivity Test was designed such that the odours selected for identification and determination of threshold levels were familiar to the Singapore population, low identification rates were observed for ASSIT when compared to the SSSIT. Low identifiability of the ASSIT could be due to the use of single chemical molecules to provide perceptions of the corresponding food items, instead of odour mixtures, as is the case for SSSIT, which has signifiers to make identification easier (Doty, Petersen, Mensah, & Christensen, 2011).

In spite of high and statistically significant coefficient of correlation for retest sessions in several studies, such as research performed by Haehner *et al.* (2009) (r_{69} = 0.86) and Hummel et al. (1997) (r_{104} = *0*.73), the reproducibility of SSSIT was not observed in this study. In contrast, despite having a smaller number of odourants in the identification test (Hummel et al., 1997; Saito et al., 2006) test-retest correlation coefficient for ASSIT, r_{20} = 0.69, was comparable to several other studies which employ similar number of odourants for identification, such as Japanese odour sticks (r_{47} = 0.77, 13 items) (Saito et al., 2006), Connecticut Chemosensory Clinical Research Centre Test (r_{104} = 0.60, 8 items) (Hummel et al., 1997), and Cross-Cultural Smell Identification Test (CC-SIT) (r = 0.71, 12 items) (Doty et al., 1996).

Therefore, ASSIT presents a valid reliable tool for examining odour identification abilities.

2.5.1.2. Threshold Tests

The reliability of the composite threshold score from ATT10 is high, indicating that under controlled conditions, the composite threshold score is reproducible. In a previous study, SSSTT performed in 64 subjects for test-retest reliability demonstrated that when retest was performed more than one month after the first test, test-retest reliability was 0.50, which is similar to our findings (Albrecht et al., 2008). Although the same chemical compound and dilution factor was used in ASSTT, the SSSTT test-retest reliability was not statistically significant.

Nonetheless, significant correlation between SSSTT and ASSTT for both test sessions suggests that the two tests yielded congruent results, and it validated that the Sniffin' Sticks preparation technique and procedure used in this study was comparable to that of the Standard Sniffin' Sticks test, even though intensities of the two preparations differed at the same concentrations of *n*-butanol. Instead, insignificant test-retest reliability for ASSTT was likely the result of the use of 12 instead of 16 dilutions, which lowered the precision of the test as compared to SSSTT.

Although the composite mean threshold score is reliable, test-retest reliability for items in the ATT10 were found to be dependent on conditions of and the length of time between the two test sessions. Satiety has been found to be an important factor for detection thresholds of odours in rats and humans

(Aimé et al., 2007; Albrecht et al., 2009), explaining its effect on test-retest reliability of several items. Influence from the time of day for test sessions for each subject could be due to varying levels of fatigue when subjects attended different time slots for the two sessions. Interestingly, the previous meal constituents may also have had an effect on low reproducibility of certain odourants, such as mushroom, smoke, cheese and onion, which tend to be odours occurring more commonly in main course meals, as compared to orange, banana, rose, cinnamon, mint and popcorn. Nonetheless, apart from cheese, threshold scores of all the odourants were not significantly different with time. Moreover, alike visual and auditory sensitivity, several researchers have found individual fluctuations in sensitivities towards odourants across time of the day and from day-to-day (Punter, 1983; Rabin & Cain, 1986; Stevens & Cain, 1987; Stevens et al., 1988). Thus, careful considerations were made for extensive testing with a sufficiently large pool of subjects in the larger population study using the Specific Sensitivity Test.

Although the composite mean threshold score was significantly higher in the second session than the first, and was especially so for orange and cheese, the difference in threshold scores was likely to be due to the subjects' familiarity with the test procedures (Rabin & Cain, 1986) and not caused by changes in the adapted Sniffin' Sticks. The stability of the odourants in the Sniffin' Sticks was supported by consistent identification rates and mean intensity ratings of the individual compounds between the sessions.

All in all, the ASSIT and ATT10 were validated and showed good quality and consistency in perceived intensity of the odour sticks for up to 5

months from preparation date, which is close to the 6-months shelf life of the Standard Sniffin' Sticks battery test, as instructed on the kit.

2.5.2. Findings of Preliminary Adapted Specific Sensitivity Test

As cognitive capabilities decrease in the elderly, and olfactory sensitivity is influenced by the ability to recognize odours (Lehrner et al., 1999), a relationship between olfaction (sensitivity) and cognition (identification) must be established to understand the actual changes in olfactory sensitivity in the elderly. Interestingly, results from the ASSIT and ATT10 indicated that, for the odour compounds used in this study, there was no clear relationship between the ability to identify an odour and threshold. The relationship, or lack of, would be ascertained when extended to a larger population.

A number of studies have established significant changes in odour identification abilities with age (Doty et al., 1984b; Kobal et al., 2000; Saito et al., 2006; Wysocki & Gilbert, 1989), including experiments performed using SSSIT (Hummel et al., 2007; Hummel et al., 1997; Katotomichelakis et al., 2007), and the same trend was observed in this study for ASSIT, but not for SSSIT, which could be the consequence of cultural differences in familiarity with the odours tested. Compared to the SSSIT, ASSIT may thus be a more sensitive test for determining the life-span decrease in identification ability for a Singapore population for the purpose of this study. Moreover, when odour mixtures are used, such as in the case of the SSSIT, if olfactory sensitivity for a component in the odour mixture has been compromised, a distorted

perception of the mixture may prevent successful identification (Doty et al., 2011), therefore making single-compound ASSIT an advantageous identification test.

The detection threshold of *n*-butanol has also been observed in several studies to increase with age (Hummel et al., 2007; Hummel et al., 1997; Katotomichelakis et al., 2007). Both SSSTT and ASSTT demonstrated the same trend, with results from ASSTT showing stronger correlation with age. In both tests, *n*-butanol threshold score had a pronounced decrease between the 51-60 and 61-70 age groups, which corresponds to a published study using *n*-butanol (Katotomichelakis et al., 2007).

Age-related changes in olfactory sensitivity are odour-specific, and are related to perceived pleasantness or unpleasantness. Results from the National Geographic Smell Survey in 1989 by Wysocki and Gilbert in the United States of America, involving 1.2 million participants, first showed that age-related deficit in olfactory sensitivity is heterogeneous across odours, concentrations and life span (Wysocki & Gilbert, 1989). More than a decade after, Konstantinidis *et al.* (2006) observed, through the SSSIT, that while some odours, such as pineapple, peppermint and cinnamon, were negatively influenced by age, certain odours, such as coffee, shoe-leather and garlic, were identified by the same extent across all age groups studied. Although the same trend was not observed in SSSIT in the current study due to the limited number of subjects from each age group, but a similar pattern was observed for ATT10 threshold scores with age. Preliminary results from citrus, fruity, floral, minty, nutty and smoky odour types represented by their odourants showed decreases with age, while spicy, earthy, rancid and savoury odour

types did not. As nominal pleasant and unpleasant stimuli have been observed to activate different areas of the brain (Grabenhorst, Rolls, Margot, da Silva, & Velazco, 2010), varying rates of olfactory loss to these stimuli may be the result of non-uniform deterioration of nose-to-brain pathways with age.

The observed trends and patterns were to be ascertained by application of the Specific Sensitivity Test on the population study.

2.6. Modification of the Adapted Specific Sensitivity Test

The preliminary study validated the Adapted Specific Sensitivity Test as an effective and reproducible method to determine the olfactory identification and threshold abilities of a healthy Singaporean population. However, before taking the Specific Sensitivity Test to a population study, some modifications were made based on the preliminary study's findings.

The final version of odourants used for Specific Sensitivity Identification test (SSIT) was adjusted according to intensity ratings in the preliminary tests. Due to the differences in psychometric functions, adjustments to concentrations were also made to ensure the odourants at respective concentrations are representative of the descriptor and at intensity levels that ensures all subjects, from young to old, are able to perceive.

In addition, concentrations of the odourants in Specific Sensitivity Threshold test were also adjusted so that the mean threshold score for each odour would be approximately 6, the median of 12 dilution sticks, taking threshold score to be equivalent to zero when the highest odourant

concentration cannot be detected. Such estimates were made according to the preliminary ATT10 mean threshold scores.

From the results in the preliminary study, the population study using the Specific Sensitivity Test also required care to ensure subject conditions were controlled as much as possible, defining the minimum number of hours before previous meal time and taking note of constituents of the previous meal.

Although stability of the filled Sniffin' Sticks used in the preliminary tests was estimated to be 5 to 6 months, a prudent approach was taken for the odourants in the Specific Sensitivity Test and shelf-life was reduced to 4 months from date of filling. Regular headspace GC-MS analysis of the filled Sniffin' Sticks was also conducted at regular time intervals in the 4 months to monitor gas phase odourant release from the tip of the Sniffin' Sticks.

Upon adjustment of odourant concentrations in the identification and threshold subtests, the Specific Sensitivity Test was applied in the extended population study.

CHAPTER 3: COMPARISON OF THE SPECIFIC SENSITIVITY THRESHOLD TEST WITH GAS CHROMATOGRAPHY-OLFACTOMETRY DILUTION ANALYSIS

3.1. Introduction

The measure of detection threshold in humans is dependent on several conditions, including the number of subjects, extent of subject training to the test procedure and odours, the type of test employed, the tool for odour delivery, test reproducibility, and statistical analysis of the results (Laing, 1983; Marin et al., 1988; Pangborn, 1981).

Despite years of olfactory testing, there is still a lack of a standard device for the delivery of odourants in research and medical diagnostics. Individual research and groups develop olfactory testing tools built for specific experimental procedures. Of the available methods of odour delivery, headspace delivery via devices, such as Sniffin' Sticks (Hummel et al., 1997)

or through scratch and sniff cards (Wysocki & Gilbert, 1989; Doty et al., 1984a), and through delivery with dedicated apparatus, such as olfactometers, are the most commonly cited in literature.

Olfactometers have been suggested to produce the best reproducible and quantitative detection threshold measurement (Laing, 1983; Marin et al., 1988; Schmidt & Cain, 2010). Modern olfactometers have been developed to control odourant concentrations, odourant mixtures, time and duration of delivery, temperature, and air flow rates for delivery of odours (Schmidt & Cain, 2010; Sezille et al., 2013; Williams, Satre, Parisot, Kurtz, & Acree, 2009). Among the types of olfactometers (Hunter, 1983; Marin et al., 1988; Shriever, Körner, Beyer, Viana, & Seo, 2011), one of such is the Gas Chromatography-Olfactometry (GC-O). The GC-O delivers single odourants at precise amounts through elution from gas chromatography. The GC-O dilution technique, CharmAnalysis^{TM}, also permits the integration of odourant delivery, data collection from subjects, and chromatographic representation of results within the ChemCharm software.

Thus, the preliminary Adapted Specific Sensitivity Threshold test (ASSTT) was compared with GC-O dilution analysis to evaluate the use of the two different tools for determining detection thresholds of human subjects.

3.2. Aims & Objectives

This study was aimed at comparing between the two detection threshold assessment tools, the Sniffin' Stick and GC-O, for the 10 odourants in the preliminary Adapted Specific Sensitivity Test and 1 odourant from the

standard Sniffin' Sticks threshold test by GC-O dilution analysis, CharmAnalysis™, with 20 subjects of various age groups from 21-80 years old.

3.3. Materials and Methods

Results from this study are part of the preliminary Adapted Specific Sensitivity Test study as described in sections 2.3.3.2 and 2.3.3.3.

Identification score of ASSIT and threshold scores of SSSTT and ASSTT were taken from the first session of the preliminary test. The same 20 subjects of the preliminary tests underwent GC-O dilution analysis during the first session of the preliminary test (Chapter 2). Order of the tests was randomized such that about half of the subjects completed GC-O dilution analysis before the Sniffin' Sticks and adapted Specific Sensitivity Test, and half after.

3.3.1. Odourants

The same odourants were used in the GC-O dilution analysis but at half the concentrations (Table 6) due to the difference in delivery of odourants to the human subjects. The odourants were mixed to form a solution with propylene glycol at respective concentrations as stated in Table 6 and the mixture was diluted further as required with propylene glycol for GC-O dilution analysis. Fresh mixtures were prepared weekly to reduce possible chemical reactions.

Table 6. Odourants and concentrations of odourants used in the Preliminary Adapted Specific Sensitivity Threshold Test and GC-O Dilution Analysis.

Odourant Name (IUPAC)	Odourant Name (Common)	Highest Threshold Test Concentration for Adapted Threshold Tests [% (v/v)]	Highest GC-O Dilution Analysis Concentration [% (v/v)]
1-Butanol	*n*-Butanol	36.0	18.0
Decanal	Decanal	0.54	0.27
3-Methylbut-1-yl ethanoate	Isoamyl acetate	1.80	0.90
2-Phenylethanol	Phenylethyl alcohol	0.30	0.15
(2*E*)-3-Phenylprop-2-enal	Cinnamaldehyde	0.405	0.2025
1-Pyrazin-2-ylethanone	Acetyl Pyrazine	0.162	0.081
(2*S*,5*R*)-2-Isopropyl-5-methylcyclo-hexanone	Menthone	28.8	14.40
Oct-1-en-3-ol	1-Octen-3-ol	0.36	0.18
2,6-Dimethoxy-4-methylphenol	4-Methyl-2,6-dimethoxyphenol	0.54	0.27
Butanoic acid	Butyric acid	2.70	1.35
2-Methyloxolane-3-thiol	2-Methyl-3-tetrahydrofuran thiol	0.0342	0.0171

3.3.2. Gas Chromatography-Olfactometry Dilution Analysis

The GC (Agilent 7890A Agilent, Palo Alto, CA, US) employed for analysis was equipped with a 30 m long, and 0.25 mm internal diameter diphenyldimethyl polysiloxane column, film thickness 0.25 μm (Agilent Technologies). Carrier gas was helium at 1.9 mL/min and column pressure of 15 psi, injector temperatures was at 250 °C. Injections were carried out in pulsed splitless mode. The temperature program was as follows: 3 minutes at 35 °C, then 10 °C/min to 160 °C, and 30 °C/min to 250 °C. Temperature of sniffing port was maintained at 250 °C, and the sniffing port was supplied with humidified air at a volumetric flow rate of 400 mL/min.

Recruited panelists underwent 4 to 6 16-minute runs of GC-O analysis with systematic dilutions of a standard mixture containing the ten odourants. The subjects were first given a demonstration on the use of ChemCharm software (Datu, Inc., USA), use of the sniffing port where volatile eluents exit the chromatographic column and are brought by a heated, humidified air stream to the subject nose. Subjects were instructed to breathe normally while sniffing the effluent from the GC throughout the run and to make three responses for each odour perceived through the sniffing port: 1) To left-click and hold the computer mouse when an odour was detected, 2) to release the mouse once the odour was no longer perceived, then 3) to choose a descriptor which best characterised the odour just perceived. The duration of each odour was dependent on the gas chromatographic retention time and oven programme. The Final Dilution Value (FDV) is the maximum dilution that the odour item was detected by GC-O. The FDV was used to determine the sensitivity of the subjects to the odourants; the larger the FDV, the more

sensitive the subject was to the odourant. The odourants were then identified by mass spectrometry. Dilutions of mixtures containing the odourants were done in factors of three, corresponding with the rest of the threshold tests.

3.3.3. Statistical Analysis

Statistical analyses were performed by SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA) for Windows. To explore threshold scores in relation to age, data was analysed with one-way analysis of variance (ANOVA). Pearson's correlation analyses were used to examine relationships between SSSTT, ASSTT, and GC-O. Alpha level was set at 0.05 for all tests. Unless otherwise stated, mean values are expressed as mean ± standard deviation (SD).

3.4. Results

3.4.1. GC-O Dilution Analysis, Standard Sniffin' Sticks, and Preliminary Adapted Specific Sensitivity Threshold Tests of *n*-Butanol

The mean threshold scores and GC-O final dilution value for *n*-butanol for all 20 subjects and each age group is summarized in Table 7. Coefficients of correlation between GC-O dilution analysis and SSSTT (r_{20} = 0.09, p = 0.72) and with ASSTT (r_{20} = 0.25, p = 0.29) are both low and not statistically significant.

Unlike SSSTT and ASSTT, GC-O dilution analysis of *n*-butanol did not show a decrease in detection threshold with age. Instead, the highest mean dilution value was that of the age group 51-60 years old, and the lowest was of

31-40, 41-50, and 71-80 years old, showing no distinctive relationship with age ($r_{20} = 0.16$, $p = 0.51$).

Table 7. Descriptive statistics of Standard Sniffin' Sticks Threshold Test, Adapted Specific Sensitivity Threshold Test for *n*-Butanol, and GC-O Dilution Analysis of *n*-Butanol and 10 odourants of the Adapted Specific Sensitivity Test.

		n-Butanol			Mean Final Dilution Value (FDV)[1]									
Age Group		Standard Threshold Score	Adapted Threshold Score	Mean Final Dilution Value (FDV)[1]	Orange	Banana	Rose	Cinnamon	Mushroom	Popcorn	Mint	Smoke	Cheese	Onion
All	Mean	7.7	5.7	1.4	4.4	4.4	6.6	4.6	3.4	7.2	3.0	6.7	6.1	4.5
N = 20	SD[2]	3.8	2.2	1.8	1.6	3.1	2.0	2.0	1.9	1.8	1.4	2.3	0.5	1.7
21-30	Mean	9.3	5.5	1.8	4.9	3.8	6.8	4.4	2.8	7.6	3.5	5.4	6.2	4.3
n = 4	SD	4.7	1.2	1.7	0.9	1.9	0.2	2.1	1.9	0.9	0.7	2.0	0.5	1.6
31-40	Mean	9.8	6.8	0.0	5.1	7.5	6.3	5.8	5.6	7.5	3.8	6.4	5.8	5.1
n = 3	SD	1.0	0.9	0.0	0.8	0.9	0.7	2.5	1.1	1.1	1.2	0.4	0.6	1.0
41-50	Mean	4.4	4.6	0.0	4.3	3.4	6.3	5.1	2.1	7.1	2.8	5.4	5.8	4.4
n = 4	SD	3.2	1.7	0.0	0.2	3.1	1.1	1.3	1.7	1.1	1.0	1.6	0.4	0.8
51-60	Mean	9.6	6.9	3.5	4.8	5.9	7.9	5.2	4.5	7.8	3.6	8.3	6.6	4.5
n = 4	SD	0.8	3.8	1.7	1.0	4.6	3.7	2.0	0.8	3.1	1.5	2.5	0.5	2.0
61-70	Mean	7.0	5.2	1.8	2.6	3.5	5.8	3.3	2.3	6.7	2.1	8.4	6.0	4.8
n = 4	SD	4.9	2.5	1.7	2.3	1.8	2.7	2.3	1.5	2.4	1.5	2.7	0.7	2.9
71-80	Mean	3.3	4.8	0.0	7.3	0.0	5.5	2.3	5.5	5.5	0.0	5.0	6.5	3.0
n = 1	SD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] Final Dilution Value (FDV) is the maximum dilution that the odour item was detected by GC-O.

[2] SD = Standard Deviation.

3.4.2. GC-O Dilution Analysis and Preliminary Adapted Specific Sensitivity Threshold Test of 10 Odourants

The final dilution value (FDV) corresponds to the maximum dilution that the subject was able to detect the odour item through the GC-O, and is regarded as the threshold of the subject for that odour compound. None of the odour items reached statistical significance for correlation between results from CharmAnalysis™ and scores from the first session of ATT10 (Table 8).

In addition, all 10 odourants did not show a significant difference in detection threshold measured by GC-O with identification proficiency measured by ASSIT.

Table 8. Pearson's correlation and statistical significance values (N = 20) between threshold levels of odour items determined by CharmAnalysis™ and Adapted Threshold Tests.

Odour Item	Correlation between CharmAnalysis™ and Adapted Threshold Test of Item, r_{20}	Statistical Significance, p
Orange	-0.19	0.42
Banana	0.14	0.57
Rose	0.21	0.37
Cinnamon	0.02	0.95
Mushroom	-0.17	0.47
Popcorn	0.33	0.16
Mint	0.16	0.51
Smoke	-0.09	0.69
Cheese	0.27	0.24
Onion	0.11	0.66

3.5. Discussion

Low and non-significant coefficients of correlation between SSSTT, ASSTT, and GC-O dilution analysis of *n*-butanol results indicate that GC-O dilution analysis was not a comparable method for the determination of *n*-butanol detection threshold for untrained panelists. The lack of compatibility between the use of Sniffin' Sticks and GC-O to determine *n*-butanol detection threshold could be the result chromatographic conditions. As *n*-butanol was the first odourant to be eluted from the GC column after the solvent, thus uncertainty as to whether *n*-butanol was part of the background odour from the sniffing port or one of the odourants may have caused subjects to miss the elution of *n*-butanol or lapse in response.

GC-O utilises a constant stream of humidified air to deliver volatiles, one by one and without anticipation, to the passive subject as and when the odourants elute from the column. Assuming normal breathing behaviour and attentiveness of the subject, the odourant is perceived. In contrast, the Sniffin' Stick requires the subjects to initiate active sniffing of the odourants in headspace from the tip of the Sniffin' Sticks upon instruction but on their own time. As a result, subjects were able to actively engage in breathing in, delivering odourants to their olfactory nares and epithelium, and were equipped with mental preparation for olfactory perception of the odourants (Bensafi, Pouliot, & Sobel, 2005). In addition, a single sniff at an increased flow rate rather than normal breathing is optimal in odour perception during threshold testing (Laing, 1983; Sobel, Khan, Hartley, Sullivan, & Gabrieli, 2000). Therefore, the difference in delivery of stimulus and sniffing behaviour differences for GC-O and Sniffin' Sticks may have accounted for low

coefficients of correlation between results from the two tests, with the latter achieving results with more accurate detection thresholds.

Despite lower variability in final dilution values from GC-O dilution analyses when compared to the threshold scores of the Sniffin' Sticks tests, the range of dilution values for GC-O dilution analyses were between 0 to 7, i.e. dilution of 2187 times, as compared to SSSTT (min. score = 0; max. score = 16, i.e. dilution of more than 43 million times) and ASSTT (min. score = 0; max. score = 12, i.e. 531441 times), thus precision is a concern when detection threshold is determined by GC-O dilution analysis. In addition, although there are no limits to the number of dilutions performed to reach detection threshold of subjects, there is a limit on the maximum concentration of odourants that may be injected into the column to prevent overloading of the chromatography column and maintain reproducibility of chromatographic runs. Therefore, GC-O dilution analysis may be better suited for measurement of detection threshold for odourants with high potency.

In terms of mobility, the use of GC-O dilution analysis is also not an ideal technique for sampling of large populations, as the technique requires a substantial amount of time to complete, with sample injection, chromatographic run time, and oven temperature programme conditions controlling the amount of time between runs.

In conclusion, the measure of detection thresholds of the 11 tested odourants via GC-O dilution analysis was not comparable to the use of Sniffin' Sticks for odourant delivery. In addition, ease of use and mobility of the Sniffin' Sticks and initiation of subject sniffing behavior for odour perception ascertained the appropriate use of Sniffin' Sticks for assessment of olfactory

capabilities of ethnic Chinese Singaporeans and Singapore Permanent Residents (PRs) in the population study.

CHAPTER 4: CHANGES IN OLFACTORY FUNCTION WITH AGE

4.1. Introduction

Through the preliminary adapted Specific Sensitivity Test (Chapter 2), we have validated the Specific Sensitivity Test for method, application, and reproducibility. Consequently, the Specific Sensitivity Test was used to assess olfactory competency of the Chinese population of Singapore at various life stages. To address both concerns about statistical significance to represent a large population such as that of the ethnic Chinese in Singapore, and to minimize variability among and within individuals (Punter, 1983; Rabin & Cain, 1986; Stevens & Cain, 1987; Stevens et al., 1988), at least 30 subjects, evenly spread across each age group as far as possible, were assessed using the Specific Sensitivity Test for every decade (21-30 years old, 31-40 years old, and so on).

4.2. Aims & Objectives

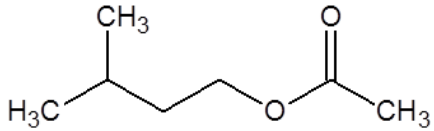
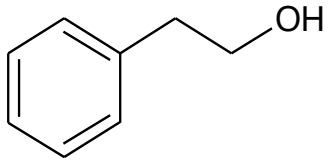
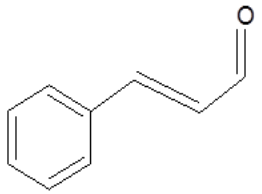
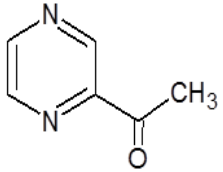
The Specific Sensitivity was used as a tool for the assessment of olfactory competency, specifically identification proficiency and threshold sensitivity, in an ethnic Chinese Singapore population.

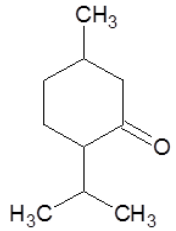
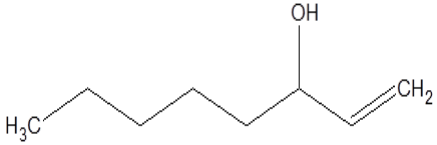
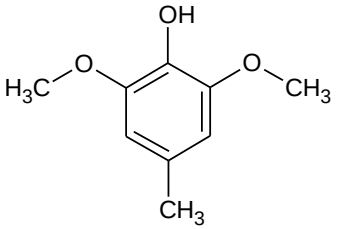
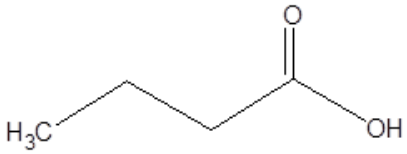
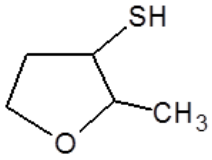
4.3. Materials and Methods

4.3.1. Odourants

The Specific Sensitivity Test consists of 10 odourants of various odour groups, molecular weights, densities, and vapour pressures (Table 9). Concentrations of the ten odourants used in the Specific Sensitivity Test were adjusted from the preliminary test as reported in section 2.5 (Table 6).

Table 9. Physical and chemical properties of odourants in the Specific Sensitivity Test.

Odourant	Chemical Structure	Molecular Weight (g/mol)	Molecular Groups	Density[1] (g/mL)	Vapour Pressure[1] mmHg at 25 °C
Decanal		156.27	aldehyde	0.83	0.207
Isoamyl acetate		130.19	ester	0.876	5.60
Phenylethyl alcohol		122.16	aromatic ring, alcohol	1.02	0.0868
Cinnamaldehyde		132.16	aromatic ring, aldehyde	1.05	0.0265
Acetyl Pyrazine		122.13	pyrazine, ketone	1.11	0.188

Menthone		154.25	monoterpene, ketone	0.895	0.256
Octenol		128.21	alkene, alcohol	0.840	0.531
4-Methyl-2,6-dimethoxyphenol		168.19	phenol, methoxy	1.10	0.005
Butyric acid		88.11	acid	0.960	1.65
2-Methyl-3-tetrahydro-furan thiol		118.2	thiol, furan	1.04	3.01

[1] Density and vapour pressure values were obtained from The Good Scents Company (n.d.).

4.3.2. Participants for Specific Sensitivity Test

Two hundred and eighty-one participants [186 females (F), 95 males (M); mean ± standard deviation age: 47.3 ± 17.5; median age: 49.0] aged 21-80 were recruited in Singapore from April 2013 to February 2014. The study samples were divided into six decades: 21-30 (n = 63; F = 43, M = 20), 31-40 (n = 48; F = 24, M = 24), 41-50 (n = 38; F = 27, M = 11), 51-60 (n = 54; F = 43, M = 11), 61-70 (n = 45; F = 25, M = 20), 71-80 (n = 33; F = 24, M = 9). All participants were Singaporean or Singapore Permanent Residents (PRs), of Chinese ethnicity, and self-reported to be generally healthy, with no current or history of major olfactory disturbance. The study was conducted with the approval of the University Institutional Review Board. All experimental procedures were explained in detail to volunteers and informed consent was obtained. Participants completed a questionnaire providing demographic information (gender, age, occupation environment, usage frequency of scented products), self-assessment of smell ability, allergies, smoking habits, diagnosed illnesses, and time of last consumed meal/beverage before commencement of the Specific Sensitivity Test.

4.3.3. Specific Sensitivity Test

Preparation and odourant presentation of the Sniffin' Sticks in the Specific Sensitivity identification and threshold tests were as described in Section 2.3.3. Any additional procedures are described in this section. Concentrations used for the Identification Test (Table 10) were selected based

on the dilutions of odourants which best reflect the desired descriptor and from results of the preliminary test (Chapter 2).

Identification test results for orange were excluded from mean identification scores in this section due to close-to-chance identification rates across all age groups (Correct Identification = 17 %, *N* = 281), thus overall identification score is the total number of correct identification of odourants' descriptors out of nine odourants. However, the composite threshold score was the mean of all ten odourants' threshold scores.

Table 10. Odourant descriptors and concentrations used in the Specific Sensitivity Test

Odourant (IUPAC)	Odourant Name (Common)	Odour Descriptor	Identification Concentration [% (v/v)]	Highest Threshold Concentration [% (v/v)]
Decanal	Decanal	Orange	1.62	1.62
3-Methylbut-1-yl ethanoate	Isoamyl acetate	Banana	2.00	0.60
2-Phenylethanol	Phenylethyl alcohol	Rose	0.90	5.40
(2*E*)-3-Phenylprop-2-enal	Cinnamal-dehyde	Cinnamon	4.000	3.645
1-Pyrazin-2-ylethanone	Acetyl Pyrazine	Popcorn	0.486	1.485
(2*S*,5*R*)-2-Isopropyl-5-methylcyclo-hexanone	Menthone	Mint	3.20	2.00
Oct-1-en-3-ol	Octenol	Mushroom	1.08	0.36
2,6-Dimethoxy-4-methylphenol	4-Methyl-2,6-dimethoxy-phenol	Smoke	14.58	14.58
Butanoic acid	Butyric acid	Cheese	2.70	2.70
2-Methyloxolane-3-thiol	2-Methyl-3-tetrahydrofuran thiol	Onion	0.040	0.0040

New pens were freshly prepared every 4 months to minimize changes in intensity due to usage of the pens. The pens were handled and stored as per described in the Sniffin' Sticks test battery to ensure linearity of gas phase odourant release at all concentrations (Denzer et al., 2014). Headspace analysis of the Sniffin' Sticks was made at regular intervals and threshold scores were also monitored over time from the date of filling. The intensity and headspace concentrations of the odourants in the Sniffin' Sticks were found not to decline within the period of study.

In the identification subtest, subjects were requested to rate the odour's pleasantness on a 9-point hedonic scale of 1 ("Extremely Unpleasant") to 9 ("Extremely Pleasant") after identification of each odourant (Table 11).

Table 11. Odourant descriptors and distractors in the Specific Sensitivity Identification test.

Odourant	Odour Type	Odourant Identification Test			
		Identifying Odour Descriptor	Distractor #1	Distractor #2	Distractor #3
Decanal	Citrus	Orange	Clove	Smoke	Apple
Isoamyl acetate	Fruity	Banana	Walnut	Popcorn	Peach
Phenylethyl alcohol	Floral	Rose	Strawberry	Peach	Mint
Cinnamal-dehyde	Spicy	Cinnamon	Vanilla	Chocolate	Honey
Acetyl Pyrazine	Malt	Popcorn	Strawberry	Coffee	Banana
L-Menthone	Minty	Mint	Rose	Grass	Garlic
1-Octen-3-ol	Earthy	Mushroom	Mustard	Clove	Chocolate
4-Methyl-2,6-dimethoxy-phenol	Smoky	Smoke	Onion	Grass	Walnut
Butyric acid	Rancid	Cheese	Fish	Bread	Ham
2-Methyl-3-tetrahydro-furanthiol	Alliaceous	Onion	Cheese	Almond	Ham

4.3.4. Statistical Analysis

Statistical analyses were performed by SPSS Version 22.0 (SPSS Inc., Chicago, IIlinois) for Windows. Age was grouped into decades (21-30 years, second decade; 31-40 years, third decade, etc.) and treated as an independent factor for analyses exploring identification and threshold scores in relation to age groups, gender, and pleasantness ratings. The data was analysed with two-factor and one-way analysis of variance (ANOVA) and post-hoc Tukey HSD tests. Pearson's correlation analyses were used to examine relationships

between age (in years) and test scores of the Specific Sensitivity Test, including pleasantness ratings. In these cases, age was used as dependent variable. Chi-squared tests were conducted to examine gender effect on identification ability of individual odourants. Alpha level was set at 0.05 for all tests. Unless otherwise stated, mean values are expressed as mean ± standard deviation of the mean.

4.4. Results

The Specific Sensitivity Test was the first olfactory performance test performed on the ethnic Chinese population in Singapore. Each subject took approximately 40 minutes to an hour and a half to complete the Specific Sensitivity Test, including demographic survey.

4.4.1. Overall Identification Ability

Subjects of Chinese descent in Singapore (186 women, 95 men), aged between 21 to 80 years were assessed for olfactory sensitivity of 10 odourants of various odour types, chemical groups, and molecular weights. Identification test results for orange were excluded from mean identification scores in this section due to close-to-chance identification rates across all age groups (Correct Identification = 17 %, N = 281), thus overall identification score is the total number of correct identification of odourants' descriptors out of nine odourants.

Results from the Specific Sensitivity Test showed significant difference in the ability to identify all odourants by age groups [$F(5,275)$ =

7.65, $p < 0.001$] (Figure 9). A significant decrease in overall identification scores was observed from subjects in the sixth to the seventh decade, while overall identification rates were comparable from the second to the sixth decade, with a slight peak in the fourth decade. Thus, there was only a weak negative correlation between individual overall identification rate and age ($r_{281} = -0.28$, $p < 0.001$).

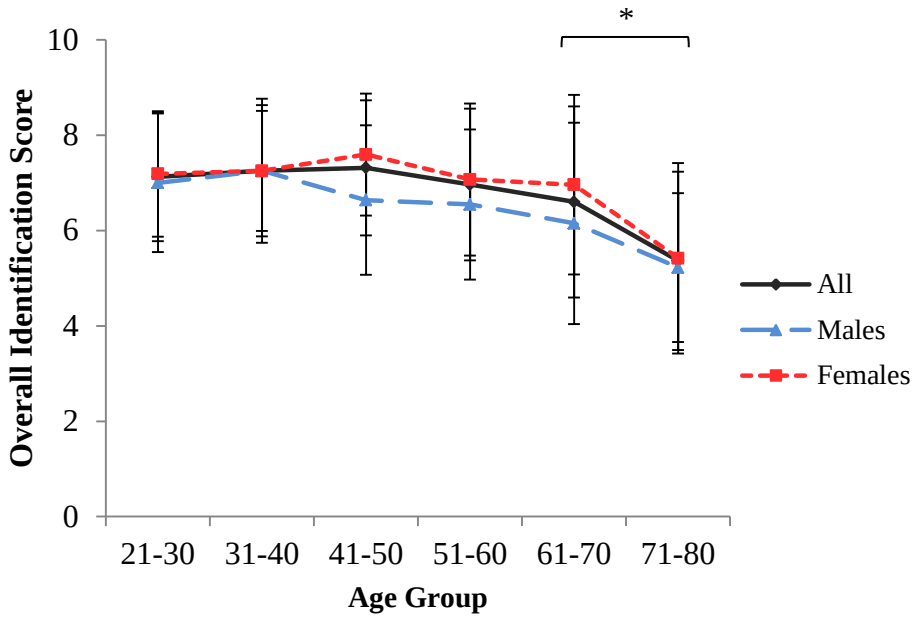


Figure 9. Mean identification scores of $N = 281$ subjects as a function of age and gender. The identification score was taken as the number of correct identification of the odourant descriptor, out of 9.
* Denotes significant difference ($p < 0.05$) between mean scores of age groups by Tukey's HSD.

4.4.2. Composite Threshold

Significant decline was observed for composite threshold scores (Figure 10), which is the mean of all 10 odourants' threshold scores, with age [$F(5,275) = 19.76$, $p < 0.001$] but no gender [$F(1,279) = 0.05$, $p = 0.82$] or interaction effects were found between gender and age group [$F(5,269) = 0.60$, $p = 0.70$]. In addition, no gender effect was observed on individual odourant

threshold scores by ANOVA ($p > 0.05$). In addition, coefficient of correlation between composite threshold scores and age was relatively high and significant at $r_{281} = -0.51$ ($p < 0.001$). Post hoc Tukey tests showed significant decrease in composite threshold scores from the second to the fifth decade before rate of decline increased, with significant decrease in sensitivity from the fourth decade to the sixth, and fifth to the seventh decade (Figure 10).

Removing age as a factor, females [Identification score (Id_{female}) = 7.0 ± 1.7] did not outperform the males (Id_{male} = 6.6 ± 1.7) significantly, both in overall identification scores [$F(1,279) = 2.68$, $p = 0.10$] and within age groups ($p > 0.05$). There was also no significant interaction effect between age groups and gender [$F(5,269) = 0.57$, $p = 0.72$].

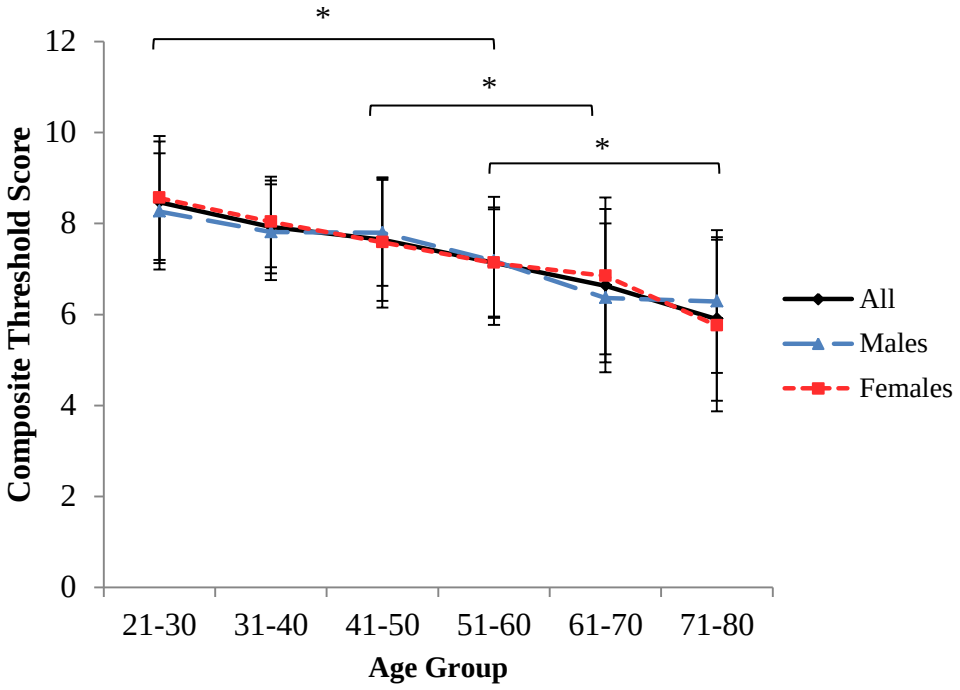


Figure 10. Composite threshold scores of $N = 281$ subjects as a function of age and gender.
* Denotes significant difference ($p < 0.05$) between mean scores of all subjects in age groups by Tukey's HSD.

4.4.3. Odourant Analysis

4.4.3.1. Identification Ability

As identification rates of orange were close-to-chance rates across all age groups, thus results for the orange odourant have been excluded from this section. Age affected successful identification rates to varying extents for the remaining nine odourants (Figure 11). Sensitivity decline with age was evident for rose throughout adulthood, while for that of onion, mint and popcorn stayed constant in the beginning decades, before decline from the fourth decade for onion and mint, and fifth decade for popcorn. Identification of cheese peaked in the third decade before falling linearly with age, whereas a dip was observed in the third decade for banana and cinnamon, before dropping again after the fifth decade. Smoke identification peaked in the sixth decade, and was lowest for the second and seventh decades. Interestingly, identification rates for mushroom did not show any significant age-dependent response.

Identification rates of rose, cinnamon, mint, smoke, cheese, mushroom, and onion were not affected by gender ($p > 0.05$), but females performed better than males for successful identification of popcorn [Correct Id_{male} = 57.9%, Correct Id_{female} = 70.4%; $\chi 2(1) = 4.42$, $p = 0.04$] and banana [Correct Id_{male} = 70.5%, Correct Id_{female} = 83.3%; $\chi 2(1) = 6.22$, $p = 0.01$].

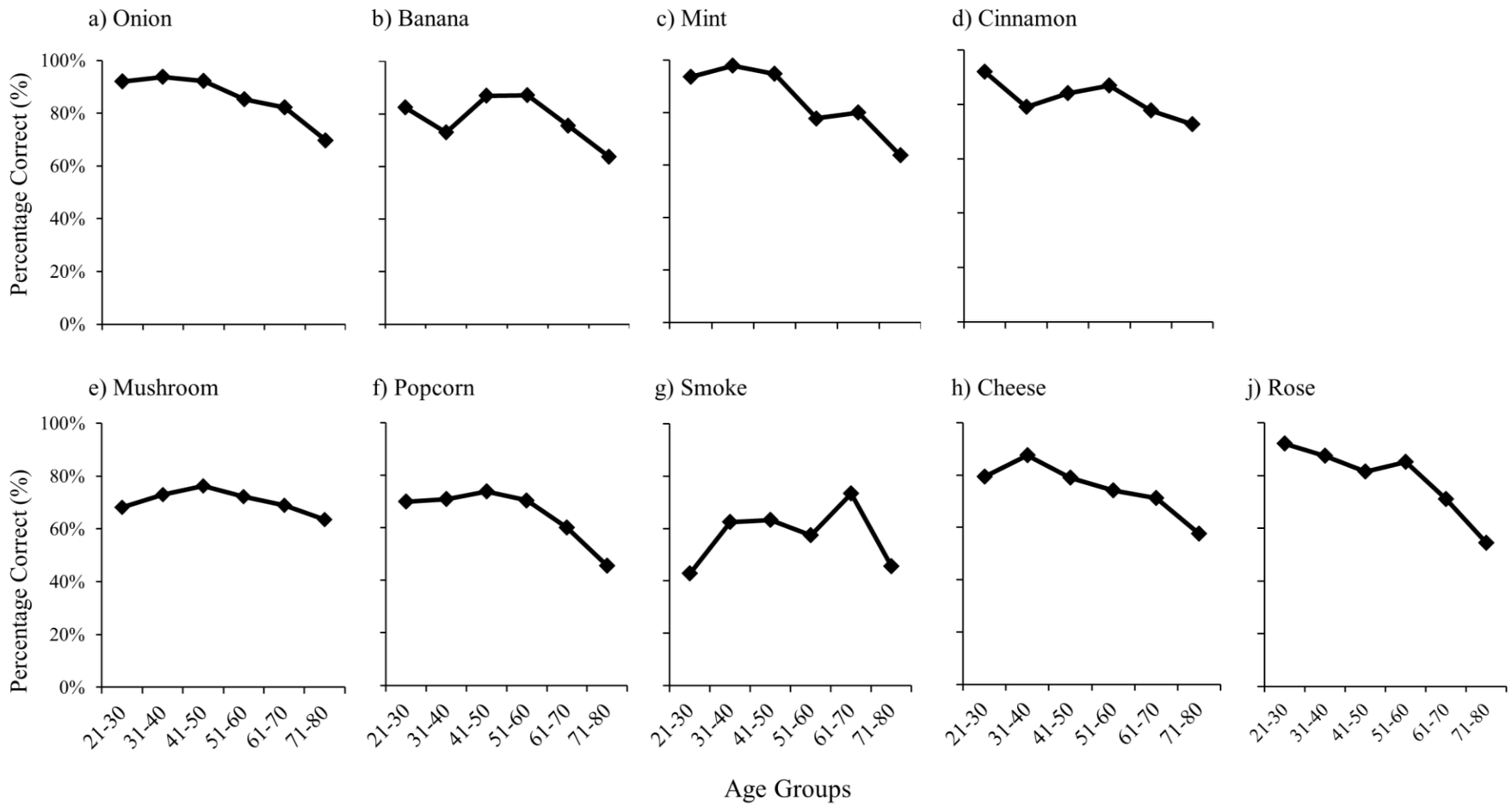


Figure 11. Percentage of N = 281 subjects correctly identified tested odourants as a function of age for: a) Onion, b) Banana, c) Mint, d) Cinnamon, e) Mushroom, f) Popcorn, g) Smoke, h) Cheese, j) Rose.

4.4.3.2. Threshold Sensitivity

Mean odourant threshold scores plotted against age groups showed onset of loss of olfactory sensitivity were also specific to odourants (Figure 12). While onion, banana, and popcorn had the most significant losses in mean threshold scores from the fifth to seventh decade and smoke from the sixth to seventh, gradual decline throughout adult life was observed for orange, mint, mushroom, and cheese. Interestingly, two odourants had the steepest losses in olfactory sensitivity in the early adult years; cinnamon had a significant loss in threshold sensitivity from the third to fifth decade, and rose had the steepest loss from the second to third decade, before further losses from the fourth to the seventh decade.

Of the 10 odourants, the largest change in detection threshold with age was that of rose, with a marked decrease by almost 2 threshold score units, which was equivalent to 9 times concentration difference, from the 2^{nd} to 3^{rd} decade, and 4.7 score units, equivalent to about 179 times concentration difference, from the second to seventh decade. The smallest change with age was onion, which did not decline until the fifth decade and only decreased by about 1 unit (3 times concentration difference) from the second to seventh decade. The remaining odourants differed from the second to fourth decade and second to seventh decade by 0.4 – 1.1 and 2.2-3.2 score units (1.5-3.3 and 12-34 times concentration difference), respectively.

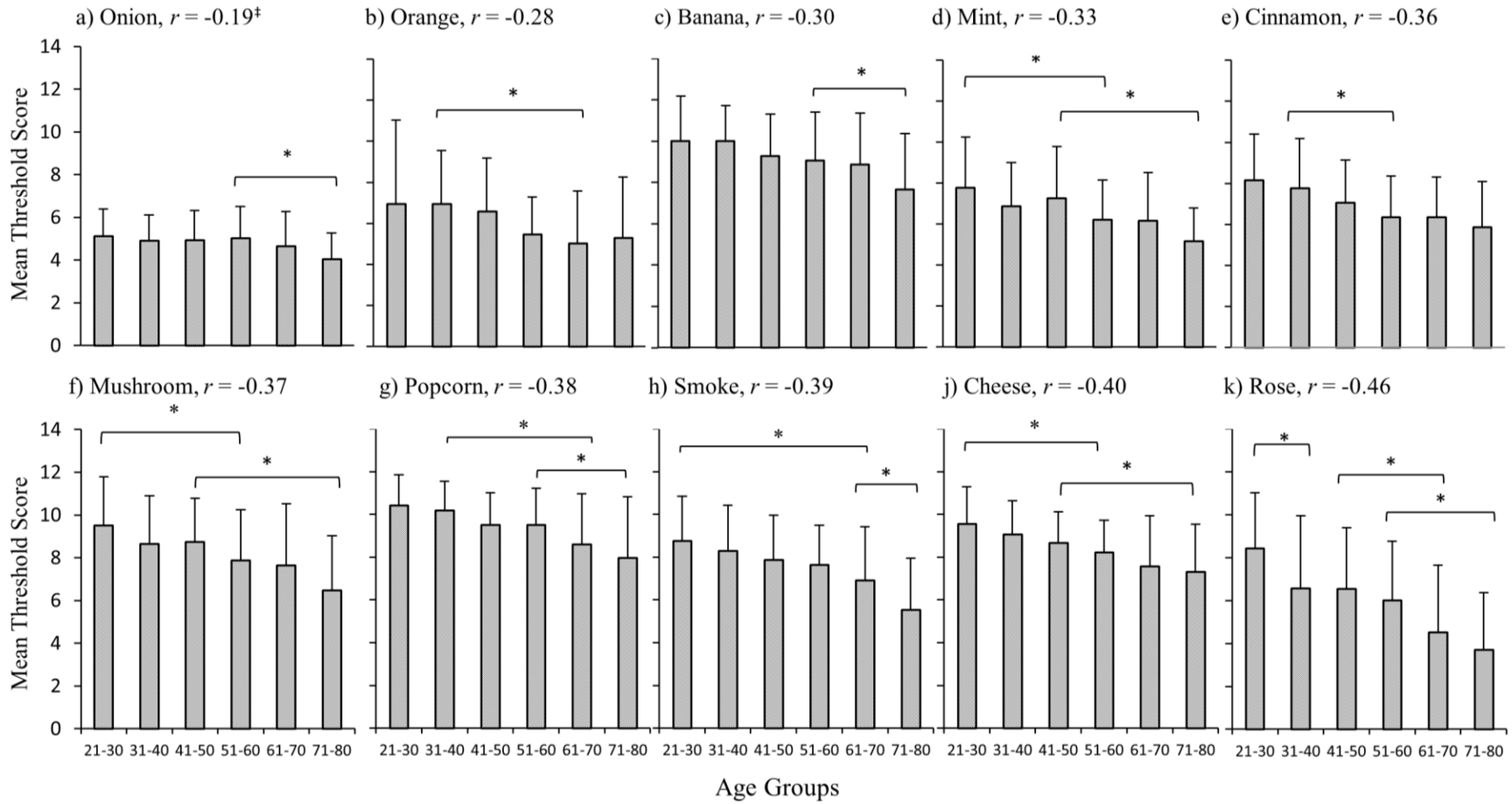


Figure 12. Mean threshold scores of odourants as a function of age for odourants: a) Onion, b) Orange, c) Banana, d) Mint, e) Cinnamon, f) Mushroom, g) Popcorn, h) Smoke, j) Cheese, and k) Rose. Standard deviations of mean values are represented by error bars on the chart.

* Denotes significant difference between mean threshold scores by one-way ANOVA

‡ Coefficients of correlation, *r*, are significant ($p \leq 0.001$) for all odourants.

4.4.3.3. Hedonics

Hedonics for each odourant was obtained after identification for the odourant at suprathreshold. The lowest mean pleasantness rating across all ages was cheese (mean ± SD = 3.6 ± 1.9) and the highest two odourants were banana (mean ± SD = 6.5 ± 1.7) and mint (mean ± SD = 6.5 ± 1.6).

Age did not have a significant effect on the perceived hedonics of 8 of the 10 odourants by one-way ANOVA ($p > 0.05$) (Figure 13). Post hoc tests of orange pleasantness ratings [mean ± SD = 4.9 ± 1.6; $F(5,275) = 2.37$, $p = 0.04$] showed significantly higher ratings for the third and sixth decades as compared to the second. Pleasantness ratings of mint [$F(5,275) = 2.77$, $p = 0.02$] peaked in the third decade and fell significantly to the seventh decade.

Of the nine odourants, pleasantness ratings had a significantly positive effect on the identification rates of banana [$F(7,273) = 4.97$, $p < 0.001$], mint [$F(8,272) = 11.36$, $p < 0.001$], and mushroom [mean ± SD = 5.1 ± 1.6; $F(8,272) = 7.07$, $p < 0.001$], negative effect for smoke [mean ± SD = 4.2 ± 1.8; $F(8,272) = 3.03$, $p = 0.003$], and identification rates peaked at pleasantness ratings of 2 and 8 for cinnamon [mean ± SD = 5.8 ± 1.9; $F(8,272) = 3.51$, $p = 0.001$] (Figure 14). Two-way ANOVA of identification rates also showed interaction effect between hedonics and age for banana [$F(30,238) = 1.85$, $p = 0.006$] and mint [$F(29,238) = 2.97$, $p < 0.001$]. For both, while identification ability of the odourants for the younger subjects is independent of the perceived pleasantness, the older subjects had higher identification rates if they rated the odourants as pleasant.

Of the ten odourants, hedonic ratings for eight of them did not have a significant effect on detection thresholds of the odourants ($p > 0.05$) (Figure 15). However, high pleasantness ratings had a negative effect on threshold scores of cheese [$F(8,272) = 1.99$, $p = 0.05$] and smoke [$F(8,272) = 2.01$, $p = 0.05$] (Figure 15). Two-way ANOVA of threshold scores also showed an interaction effect between hedonics and age for cheese [$F(36,231) = 1.78$, $p = 0.007$]. Contrary to identification rates, it was found that threshold sensitivity of the elderly was lowered if they rated the cheese odourant highly.

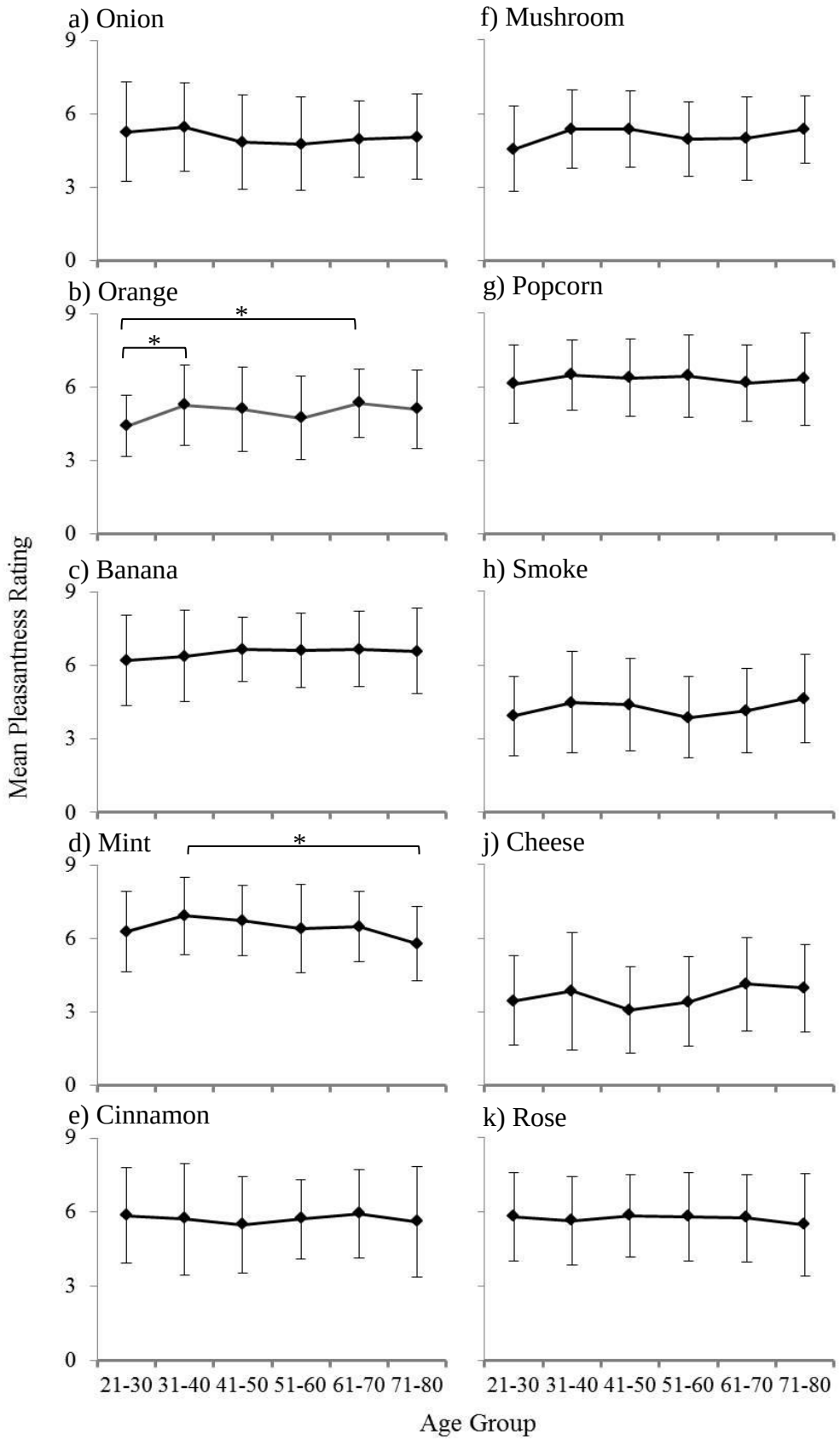


Figure 13. Mean pleasantness ratings as a function of age for odourants: a) Onion, b) Orange, c) Banana, d) Mint, e) Cinnamon, f) Mushroom, g) Popcorn, h) Smoke, j) Cheese, and k) Rose. Standard deviations of mean values are represented by error bars on the chart. * Denotes significant difference between mean threshold scores by one-way ANOVA.

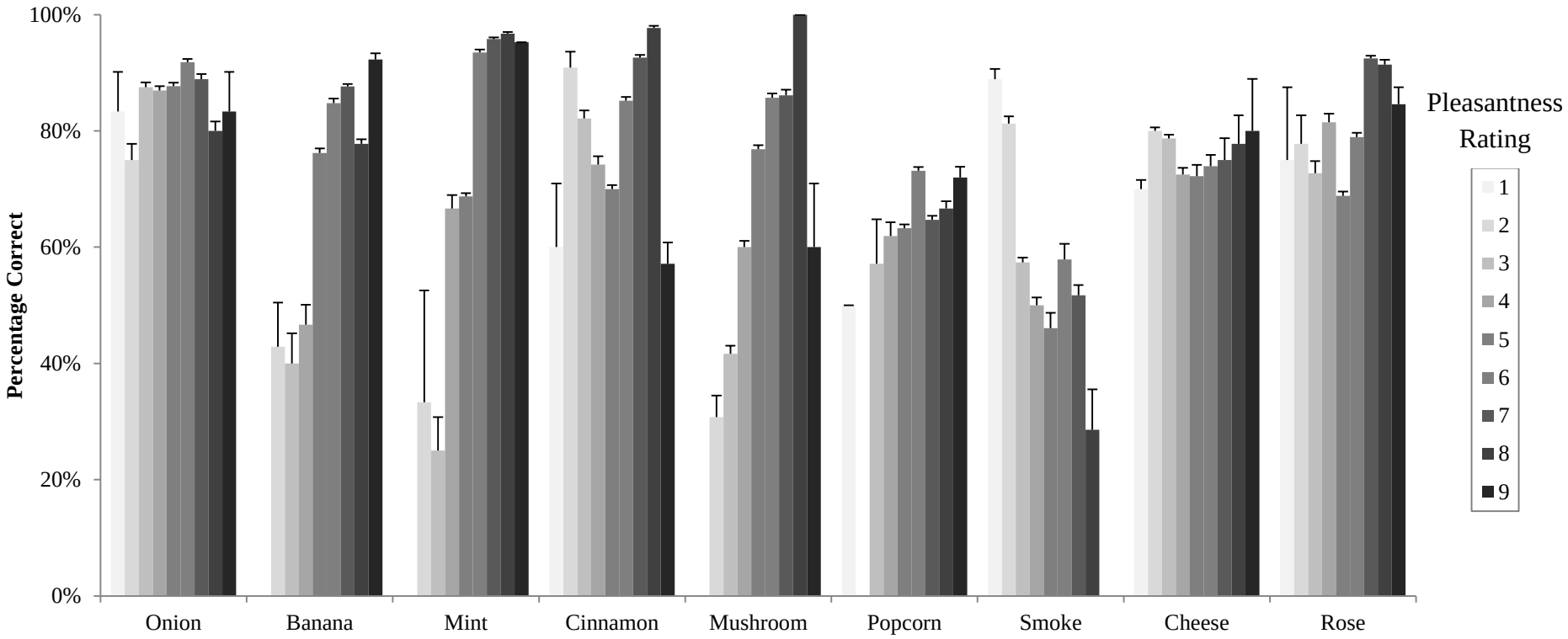


Figure 14. Identification rates expressed as percentage of correct identification as a function of pleasantness rating for nine odourants. Standard errors of mean (SEM) values are expressed as error bars in the bar chart. SEM values for $n \leq 2$ are not represented in the figure.

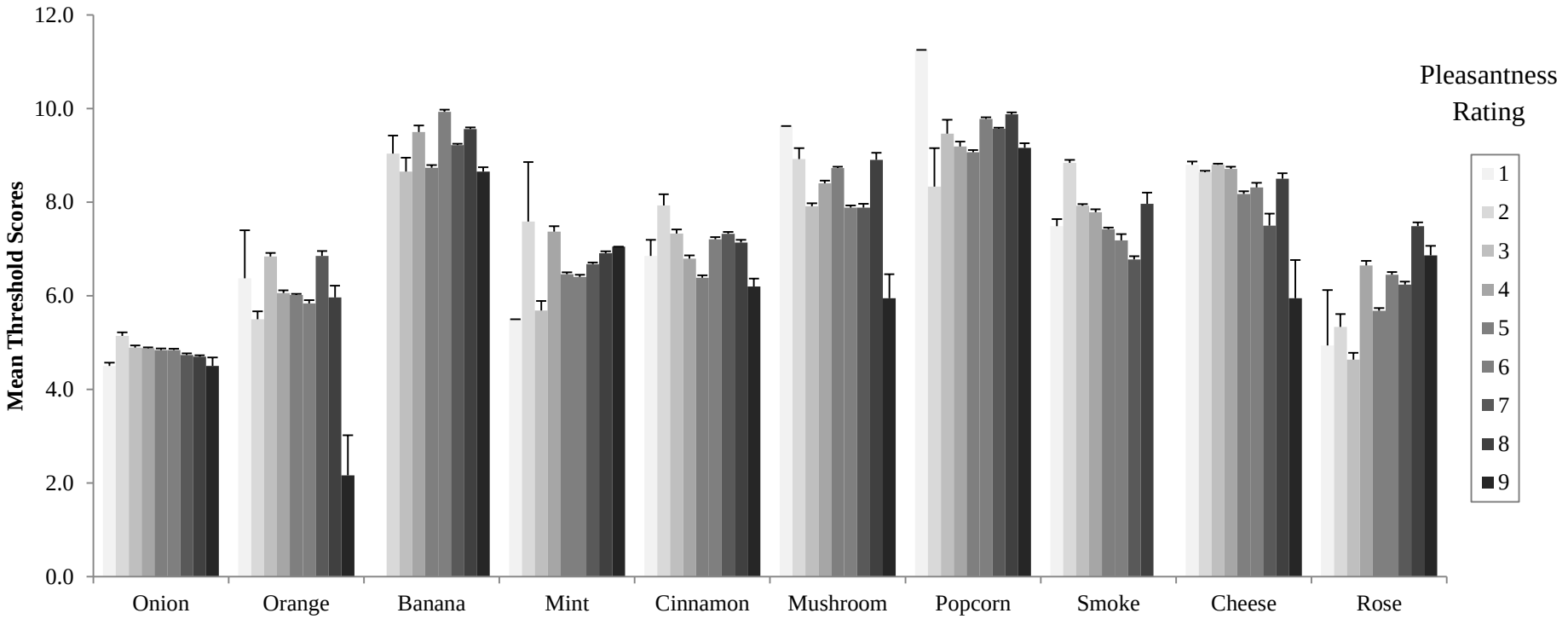


Figure 15. Mean threshold scores as a function of pleasantness rating for ten odourants. Standard errors of mean (SEM) values are expressed as error bars in the bar chart. SEM values for $n \leq 2$ are not represented in the figure.

4.4.3.4. Other Factors Affecting Olfactory Function

Regression analysis was performed for factors: 1) self-reported nose sensitivity (n = 55), 2) allergies (n = 55), 3) medical conditions with long term medication [arthritis (n = 14), diabetes (n = 14), hypertension (n = 45), heart disease (n = 4), high cholesterol (n = 31)], 4) smoke status [smoker (n = 8), used to smoke (n = 8), and don't smoke] and 5) self-reported olfactory abilities (mean rating = 3.47 ± 0.78 on a numerical scale of 1 to 5). The factors were not found to affect identification rates and threshold sensitivity ($p > 0.05$).

4.5. Discussion

Our study tested individual subjects' olfactory functions with the aim to make inferences about age-related changes in identification ability and threshold detection performance towards single odourants across participants of a wide range of ages from both genders.

4.5.1. Identification Proficiency with Age

Previous studies have demonstrated that ageing affects identification and discrimination abilities of odour mixtures (Doty et al., 1984; Schiffman 1997). The use of single odourants which are key aroma compounds in the tested odours at suprathreshold, such as isoamyl acetate in banana, minimized odour perception dependence on combination of various odourants in order to achieve successful identification, eliminating possible interaction effects such as enhancement and suppression. Although identification of single odourant is unique to this study, the observed changes in composite identification ability

with age were similar to studies using mixtures of odourants (Doty et al., 1984; Katotomichelakis et al., 2007; Schubert, Cruickshanks, Klein, Klein, & Nondahl, 2011; Sorokowska et al., 2014), exhibiting age-related decrease in overall identification rates throughout adulthood, peaking in the 3rd decade or 4th decades, and decreases most significantly from the sixth to seventh decade. As odour identification is dependent on prior exposure and familiarity to the odours (Zucco et al., 2014), and correlated to memory and vocabulary abilities (Hedner et al., 2010; Larsson et al., 2004), thus identification proficiency may increase with age and experience from the 2nd decade and peak in the 3rd and 4th decades, before age-associated decline in olfactory abilities.

More importantly, looking at identification rates for individual odourants, our results confirmed the National Geographic Smell Survey's findings on odourant-specific losses in identification ability with age. While identification rates for certain odourants fell with increasing age, others remained uniform throughout life.

Interestingly, identification of smoke-like 2,6-dimethoxy-4-methylphenol showed the largest contrast from the age-dependent decline in identification proficiency for other odourants, with increasing percentage of correct identification with age up to the sixth decade. The age response curve of 2,6-dimethoxy-4-methylphenol was dissimilar to that of eugenol (4-allyl-2-methoxyphenol), which was approximately constant throughout lifetime before dipping in the sixth decade and accelerating to the eighth. Even though eugenol only differed from 2,6-dimethoxy-4-methylphenol by one less methoxy group and an allyl group instead of methyl side chain at C4 (Wysocki and Gilbert 1987), identification rates of 2,6-dimethoxy-4-methylphenol

demonstrated unprecedented discovery of increased identification proficiency with age in later adult years. Further research needs to be conducted to ascertain if such an observation is the result of increased exposure and familiarity with smoke-like odours in the middle-aged years, as this might help in understanding the selectivity of olfactory receptors.

4.5.2. Threshold Sensitivity with Age

Composite threshold elevation was more sensitive to ageing than decline in identification ability, beginning earlier in adult life as observed in previous studies (Wysocki & Gilbert, 1989; Hummel et al., 2007). Due to the diagnostic nature and/or collection of population normative values in published olfactory tests which studied the changes in olfactory functions with age, the detection threshold of an odourant, such as phenyl ethyl alcohol and *n*-butanol, is used to determine the threshold sensitivity of subjects in the study (Hummel et al., 2007). However, as demonstrated from this study, threshold sensitivity changes with age are odourant-specific and thus necessitate the use of more than one odourant to provide a true estimate of the changes in overall threshold sensitivity of subjects with age. To date, the combination of up to 10 odourants' detection thresholds to understand overall threshold sensitivity with age has not been reported in literature. Linear decline in composite threshold sensitivity with age is first reported here.

The olfactory system remains plastic throughout life (Ma et al., 2014; Tsai et al., 2014). In spite of the resilience of the human olfactory system with age, retaining capacity for neurogenesis and recovering well anatomically after

damage, deterioration of olfactory sensory neuron odour selectivity at various stages of adult life would contribute to different initiation and extents of decline in olfactory thresholds to tested odourants (Loo et al., 1996; Larsson et al., 2012). Observed losses in threshold sensitivity of some odourants from the early adult years demonstrated that loss in sensitivity does not begin with a sudden onset in the elderly years. This was found to be so especially for detection thresholds of rose-like odourant phenylethyl alcohol, which fell significantly from as early as in the 2^{nd} to 3^{rd} decade of life.

In attempts to understand how odourants may present different rates of olfactory loss with age, Sinding et al. (2014) found a relationship between the molecular weight of odorants and increase in detection thresholds with age, using 150 g/mol as the upper limit of light molecules, and lower limit of heavy molecules. Detection thresholds were elevated only for heavy molecules in the older subjects (50 to 70 years old) as compared to younger subjects (18 to 30 years old). However, we did not observe the same effect in the ten odourants of the Specific Sensitivity Test, of which three molecules are above molecular weights of 150 g/mol and the smallest molecule, butyric acid (M_r = 88.11 g/mol) fell at equal or larger extents from the age groups 21-30 to 50-70.

In addition, other physical properties of the odourants, such as boiling points, vapour pressure, and density did not have consistent effects on either identification ability or threshold sensitivity.

4.5.3. Effect of Hedonics on Olfactory Functions

Unlike the observations made by Joussain et al. (2013) and in the National Geographic Smell Survey (Wysocki & Gilbert, 1989) on changes in perceived pleasantness of odours and odourants with age, there were no significant differences in the hedonic ratings with age, for unpleasant or pleasant odours apart from that of mint, where perceived pleasantness declined gradually from the 3rd to 7th decade. Scale usage of the three studies was similar; the National Geographic Smell Survey (Wysocki & Gilbert, 1989) utilized a 5-point scale, while subjects in our study and Joussain et al. (2013) rated the odourants on a 9-point hedonics scale, eliminating concerns of differences in limitations of scales (Lim, 2011). Nevertheless, respondents of Asian ethnic consumers have been observed to utilize the middle values of the 9-point hedonic scale, while the American consumers use the extremes of the scale more often (Yeh et al., 1998). As a result, changes in hedonics towards odourants with age by ethnic Chinese Singaporeans and PRs may not be as apparent as those of the Americans and European subjects.

Although results of this study did not present a consistent relationship between the perceived pleasantness of odourants, identification rates, and age, it was worthwhile to note that the two odourants regarded as the most pleasant had higher rates of identification by the older age groups, but not for the young. This result was contrary to the findings of Konstantinidis and co-workers (2006), where it was found identification of pleasant odours was sensitive to ageing but identification of unpleasant odours stayed constant with age. The differences in findings may stem from the fact that perception of odours and their pleasantness are shaped by past and present experiences, and

frequency of exposure to the odour (Davis, 2004; Wilson, 2003). Nevertheless, researchers have also found a relationship between the physicochemical properties of odourants and their perceived pleasantness (Khan et al., 2007). Therefore, there may be a predisposition for pleasant or unpleasantness of odours regardless of culture, exposure, and experiences.

The left orbitofrontal cortex is activated to a larger extent when exposed to odours which are unpleasant than pleasant (Royet et al., 2001), and because the left orbitofrontal cortex has been observed to be activated in emotional, visual, auditory, and gustatory stimuli (Royet et al., 2000; Zald et al., 1998), findings by Royet et al. (2001) lends support to the emotional aspects of olfaction.

Due to the nature of the Specific Sensitivity Test, hedonic ratings were obtained immediately after forced-choice identification of each odourant. High coefficients of correlation have been observed between perceived intensity, familiarity, and hedonic strength of odourants (Distel et al., 1999). Recognition of an odourant and drawing on previous experience may increase its perceived hedonic strength, as in the case of banana, mint, and mushroom. However, the converse may also be true for smoke, as smoke-like odours tend to associate less strongly with positive images, but instead with images such as cigarette smoke or the burning wood, which are regarded as unpleasant to most, thus resulting in the negative effect of hedonics on identification rates.

The relationship between hedonics and detection threshold has not been investigated in literature, possibly due to the changes in perception of odourants at different concentrations. For instance, odour descriptors used for mercaptans can range from “fruity” to “meaty” while odour descriptors for

ketones can vary from “caramellic” to “raspberry-like”, depending on concentrations of the odourants and odour memory of the evaluator (Rowe, 2011). In fact, it was observed during the Specific Sensory threshold test sessions where subjects had vastly different impressions of and perceived pleasantness for the odourants at the different concentrations. Nevertheless, for the purpose of understanding how hedonic rating may affect detection threshold of an odourant, the pleasantness ratings for comparison with threshold scores were taken at suprathreshold concentrations of the identification subtest.

Perceived pleasantness only had a negative relationship with threshold scores for cheese, which was also the least pleasant odour of the ten. While perceived pleasantness of cheese did not have an effect on threshold scores for the younger subjects, older subjects who rated the cheese odourant as pleasant also had lower threshold scores for the odourant. Such an observation may be due to the decreased sensitivity of the older subjects for the otherwise pungent odourant butyric acid, thus contributing to high pleasantness ratings (Joussain et al., 2013).

4.5.4. Other Factors on Olfactory Functions

Identification rates and thresholds were not significantly higher for the females than the males in this study despite superior identification ability by women subjects observed in previous studies (Corwin et al., 1995; Katotomichelakis et al., 2007; Wysocki & Gilbert, 1989). The distinction has been attributed to gender differences in verbal skills, hormones, anatomy, and

physiology (Doty et al., 1985; Larsson et al., 2004). Detection thresholds have also been found to be less sensitive to gender than identification ability (Cometto-Münez & Abraham, 2010; Punter, 1983), lending support to the contribution of female superiority in episodic odour memory mediating higher proficiency in identification ability, and not an effect of physiological differences (Öberg, Larsson, & Backman, 2002). Familiarity to the odourants tested may also play a part as women increase olfactory sensitivity with repeated exposure faster and to a larger extent than men (Dalton et al., 2002). Due to the short time exposure and low concentrations of tested odourants in this study, repeated exposure was not of concern.

In Singapore, prevalence of diabetes amongst Singapore residents aged 18 – 69 increased in 2010 (11.3%) as compared to 1992 (8.6%), with the rise being attributed to population ageing (MOH, 2010). In addition, 29.1% and 53.4% comprising of those aged 60 – 69 were diagnosed with diabetes and hypertension, respectively (MOH, 2010). Although patients with medical conditions such as diabetes have been observed to suffer from impaired identification ability (Weinstock, et al., 1993), diabetic participants of the Specific Sensitivity Test did not present lower identification proficiency or threshold sensitivity as compared to the rest of age-matched counterparts.

Long term drug usage for medical conditions, such as hypertension and high cholesterol, have been associated with disturbances of the chemosenses as a result of nerve depositions and altered ion influxes (Ackerman & Kasbekar, 1997). In addition, allergic rhinitis has also found to contribute to olfactory impairment, regardless of age (Apter, Gent, & Frank, 1999). However, self-reported medical conditions and allergic rhinitis did not have an

effect on identification proficiency and threshold sensitivity in this study. As participants of this study were generally healthy, community-dwelling subjects not suffering from dementia or other mental illness, and subjects with medical conditions were medicated and monitored, therefore their olfactory functions may not have been affected to as large an extent as in persons with protracted medical problems.

Nevertheless, most studies on olfactory function have been conducted in Europe and the United States, while Singapore is characterized by year-long tropical climate. Considering possible effects of environmental conditions, cultural, and dietary differences, data from this study may also vary from data of other settings.

4.5.5. Evaluation of the Specific Sensitivity Test

Fatigue and adaptation of human olfactory receptors to the odourants (Cheesman & Mayne, 1953; Ekman, Berglund, Berglund, & Lindvall, 1967; Peng, Jaeger, & Hautus, 2014) were of concern due the high quantity and repetitive nature of trials in the Specific Sensitivity threshold subtest. While due diligence was made to ensure that subjects were provided with plain water and frequent rests, performance decline from lack of motivation as a result of the long test sessions may nevertheless occur. The sequence of detection threshold testing for the 10 odourants was randomized for each subject to reduce any possible bias.

Rowe and Khan (1987) noted that research on ageing placed a large emphasis on differences between age groups, while ignoring the large

variation present within age groups. To eliminate generalized age groups which encompass subjects of a wide age range, such as “the young” and “the old”, this study aimed to look at the changes decade-by-decade to be able to track precise points at which olfactory functions change with age. In spite of high variability found for detection thresholds of individuals in previous studies (Punter, 1983; Rabin & Cain, 1986; Stevens & Cain, 1987; Stevens et al., 1988), standard deviation values of mean threshold scores were within 1.21 to 2.89, such that 95 % of each age group only fell within 1 to 3 orders of magnitude for each odourant, depending on the odourant and age group, demonstrating relatively low variability, even for elderly age groups.

As a result, we deduced that a statistically sound number of subjects were recruited for each age group in this study to represent the general ethnic Chinese population of Singapore. In addition, it is believed that care taken to ensure conditions of the Specific Sensitivity Test were kept consistent across subjects, such as the application of scented products and food and beverage consumption before the test, and for subjects, such as ensuring the test location was kept odourless and at a constant temperature, also contributed to low variability in the data.

The ability to identify an odour or odourant is frequently used as a primary measure for olfactory function of an individual. However, in the everyday consumption and enjoyment of food and detection of off odours, in food or from the environment, the ability to identify the odour is of lesser priority than the ability to detect these odours. Although detection thresholds are not absolute representatives of the individual odourants’ perceived intensities in mixtures (Cain, 1969; Cain, de Wijk, Nordin, & Nordin, 2008),

detection threshold assessment was used as an estimate of the impact heterogeneous loss in olfactory abilities towards different odourants may impact the perception of odour mixtures. Moreover, the intensity of an odourant out of a mixture and within a mixture is unpredictable and may differ to a large extent due to mixture interaction effects of suppression and enhancement (Grabenhorst et al., 2011). Therefore, it is hoped that the determination of detection thresholds with age in this study illuminates another piece of the puzzle to the changes in odour and flavour perception with age.

Our finding on overall identification ability for a set of individual odourants of the Chinese Singaporean and PRs with age using the Specific Sensitivity Test is in agreement with published literature on age-associated changes in identification proficiency of odour mixtures across the world. In addition, this study was also the first to demonstrate linear loss in composite detection thresholds with age by combination of individual detection thresholds of 10 single odourants.

More significantly, our results proved that both identification ability and detection thresholds decline at varying rates with age and these changes are odourant-specific, confirming findings of the landmark study, National Geographic Smell Survey (Wysocki and Gilbert 1987). Of the ten odourants in the Specific Sensitivity Test, we did not find pleasantness ratings and molecular weight of the odourants to be contributing factors to the varying rates of loss.

Excluding interaction effects in an odour mixture, our findings illustrated that in a mixture containing two or more of the tested odourants,

subjects in their 20s will be able to perceive all odourants whilst subjects in the middle or elderly years will perceive a different mixture with less odourants, thereby contributing to distortion in mixture perception at various stages of adult life. Odourant-specific loss of detection thresholds throughout adult life span could explain discrimination ability decline of odour mixtures from middle aged years (Cain et al. 1990) and unsuccessful compensatory strategies taken to overcome olfactory decline for the elderly using intensification of odour mixtures or introduction of ingredients and odour mixtures to the prepared foods (Schiffman and Warwick 1993; Griep et al. 1997; Koskinen et al. 2003a; Koskinen et al. 2003b; Koskinen et al. 2005; Koskinen et al. 2005; Laureati et al. 2003).

CHAPTER 5: RELATIONSHIP BETWEEN IDENTIFICATION ABILITY AND THRESHOLD SENSITIVITY

5.1. Introduction

Odour detection threshold, unlike odour identification ability, is not affected by cognitive factors such as executive functioning, semantic memory, and episodic memory (Hedner et al., 2010; Larsson et al., 2004), and likely to require low-level perceptual functions with low to no cognitive demands (Cain et al., 1990).

It is thus pertinent to gain insight on the extents of loss in olfactory functions, specifically in identification and threshold sensitivity, for single odourants with age in order to understand the interaction between identification ability and detection thresholds.

5.2. Aims and Objectives

This study aimed to elucidate the relationship between identification proficiency and detection thresholds for a set of single odourants as a function of age in Singapore.

5.3. Materials & Methods

Results from this study are part of the population study using the Specific Sensitivity Test as described in Chapter 2. Materials and methods are as described in Section 4.3.

5.3.1. Statistical Analysis

Statistical analyses were performed by SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA) for Windows. Age was grouped by 20 years interval (21-40 years, young; 41-60 years, middle; and 61-80 years, elderly) and treated as an independent factor for analyses exploring identification and threshold scores in relation to age groups and gender.

The data was analysed with two-factor and one-way analysis of variance (ANOVA) and post-hoc Tukey HSD tests. Linear regression analyses were carried out on Specific Sensitivity Test scores to determine the interaction effect of age on the relationship between identification and threshold scores. Alpha level was set at 0.05 for all tests. Unless otherwise stated, values are expressed as mean ± standard deviation.

5.4. Results

5.4.1. Overall Identification Ability

As identification rates of orange were close-to-chance rates across all age groups, thus results for the orange odourant have been excluded from this section. The overall identification score is the total number of odourants correctly identified out of a possible nine odorants.

Results from the Specific Sensitivity Test (Table 12) showed significant difference in overall identification ability by age groups [$F(2,278) = 12.34$, $p < 0.001$]. Identification ability generally decreased with age, but significant decline was only found between the middle and elderly age groups.

Removing age as a factor, females [Identification score (Id_{female}) = 7.0 ± 1.7] did not outperform the males (Id_{male} = 6.6 ± 1.7) significantly, both in overall identification scores [$F(1,279) = 2.68$, $p = 0.10$] and within age groups ($p > 0.05$). There was also no significant interaction effect between age groups and gender [$F(2,275) = 0.73$, $p = 0.48$].

5.4.2. Composite Threshold

Significant decline was observed for composite threshold scores (Table 12), which is the mean of all nine odourants' threshold scores, with age [$F(2,278) = 42.73$, $p < 0.001$]. Post hoc Tukey tests showed significant losses in composite threshold scores among all three groups. However, there was no gender [$F(1,279) = 0.12$, $p = 0.73$; Threshold $Score_{female}$ = 7.43 ± 1.66, Threshold $Score_{male}$ = 7.39 ± 1.51] or interaction effects between gender and age [$F(2,275) = 0.55$, $p = 0.58$].

Table 12. Descriptive statistics of Specific Sensitivity Identification and Threshold test scores obtained from $N = 281$ subjects.

Age Group		Overall Identification Score	Composite Threshold Score	Threshold Score								
				Onion	Banana	Cinnamon	Mint	Mushroom	Popcorn	Smoke	Cheese	Rose
Young	Mean	7.2	8.38	5.01	10.00	7.99	7.40	9.14	8.55	8.55	9.34	7.63
21-40 Years Old	Standard Deviation	1.4	1.21	1.25	1.97	2.32	2.35	2.29	2.11	2.11	1.69	3.10
$N = 111$	Minimum	4.0	5.00	2.75	3.50	4.50	4.25	2.25	2.00	2.00	6.00	0.00
	Maximum	9.0	11.17	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Middle-aged	Mean	7.1	7.51	4.98	9.15	6.65	6.66	8.23	7.73	7.73	8.42	6.23
41-60 Years Old	Standard Deviation	1.5	1.27	1.44	2.19	2.05	2.22	2.26	1.96	1.96	1.49	2.79
$N = 92$	Minimum	3.0	4.64	0.00	4.75	0.00	3.25	2.50	3.50	3.50	5.00	0.00
	Maximum	9.0	10.83	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Elderly	Mean	6.1	6.45	4.40	8.36	6.15	5.75	7.14	6.33	6.33	7.46	4.18
61-70 Years Old	Standard Deviation	2.0	1.78	1.49	2.62	2.07	2.11	2.80	2.55	2.55	2.30	2.96
$N = 78$	Minimum	1.0	1.28	0.00	1.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maximum	9.0	9.25	9.50	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00

5.4.3. Overall Odourant Identification Rates & Threshold Scores

To compare individual odourant identification ability and threshold sensitivity across ages, mean threshold scores for each odourant were compared between subjects who correctly identified the odourant and those who identified incorrectly (Figure 16). Although threshold scores for all the odourants were slightly elevated for subjects who correctly identified the odourants than those who did not, the differences were only significant for four of the nine odourants [Mint: $F(1,279) = 5.16$, $p = 0.02$; Mushroom: $F(1,279) = 10.12$, $p = 0.002$; Popcorn: $F(1,279) = 7.83$, $p = 0.005$; Rose: $F(1,279) = 27.98$, $p < 0.001$], indicating a possible but odourant-specific relationship (Wysocki and Gilbert 1989).

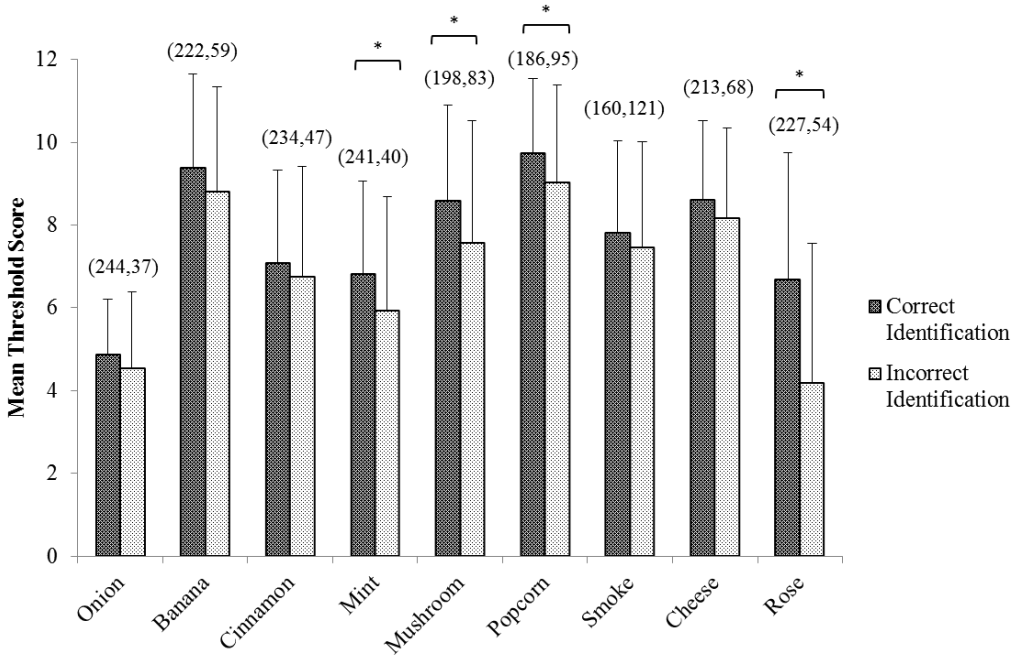


Figure 16. Mean threshold scores as a function of successful identification of individual odourants.
Values in brackets correspond to the number of subjects who correctly and incorrectly identified the odourant.
* Denotes significant difference between mean threshold scores by one-way ANOVA.

5.4.4. Odourant Identification Rates & Threshold Scores as a Function of Age

Comparing specific odourant identification ability and threshold sensitivity as a function of age, mean threshold scores for each odourant were compared between subjects who correctly identified the odourant and those who identified incorrectly in each age group (Figure 17). Significant differences were found between mean threshold scores of correct and incorrect identification for popcorn, smoke, cheese, and rose, but at inconsistent age groups. Thus, there was no clear relationship observed between the identification ability and threshold scores of the odourants with age demonstrated by the age-response curves (Figure 17).

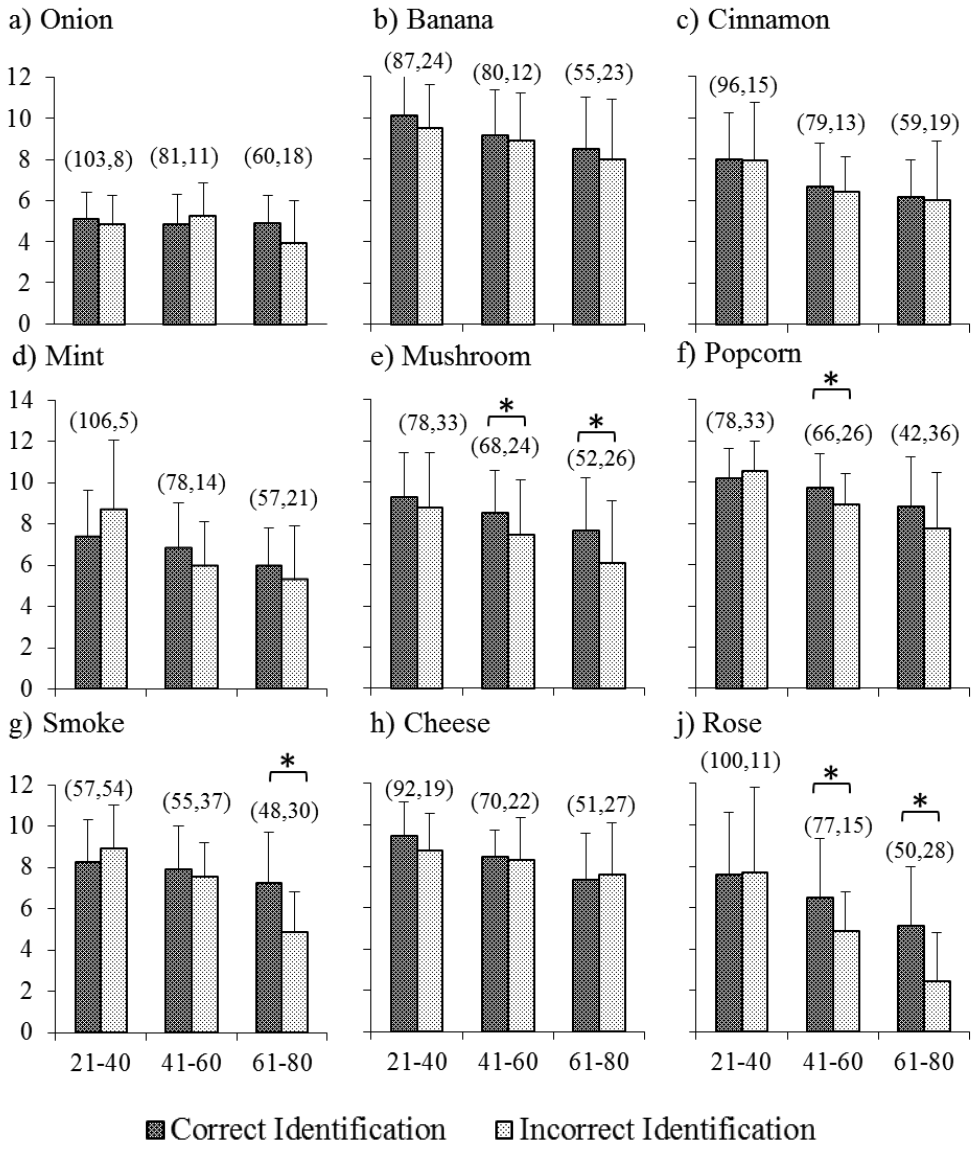


Figure 17. Mean threshold scores of young, middle-aged, and elderly groups as a function of successful identification of individual odourant: a) Onion, b) Banana, c) Cinnamon, d) Mint, e) Mushroom, f) Popcorn, g) Smoke, h) Cheese, and j) Rose.
Values in brackets correspond to the number of subjects who correctly and incorrectly identified the odourant in each age group.
* Denotes significant difference between mean threshold scores by one-way ANOVA.

5.4.5. Regression Analysis of Olfactory Functions

The influence of age on the relationship between overall ability to identify odourants and threshold sensitivity of specific odourant was explored by linear regression. Regression analyses were conducted on each odourant and the composite threshold score using the models of dependent variable mean threshold score, independent variables overall identification score (Id) and age groups (age), and interaction term overall identification score * age group (Id*age) with reference to the first age group (21-40 years old) (Table 13).

The regression models analysed accounted for 10.0 to 36.1 % of the mean threshold score variances and all predictors were statistically significant ($p < 0.001$) for composite and individual odourants' mean threshold scores. Regression coefficients of the overall identification score consisted of negative to very low values and were not statistically significant.

More importantly, age moderated the relationship between overall identification ability and threshold sensitivity for all but one of the tested odourant. While interaction coefficients of the middle-aged group, with reference to the young age group, were mostly not significant, they were positive and ranged from $\beta = 0.07$ ($p = 0.81$) for mushroom to $\beta = 0.63$ ($p = 0.05$) for mint. Notably, with increasing age, the overall identification ability had statistically significant and stronger positive relationship with threshold sensitivity, as exhibited by increments in interaction coefficients from the middle-aged to elderly groups. The influence of age on the relationship between overall identification ability and threshold sensitivity was not uniform across all odourants, with some showing increasing age influence throughout

adulthood, such as onion, popcorn, cheese, and rose, whereas some showed only late adulthood age influence, such as mushroom and smoke. In contrast, age influence only increased marginally for banana, cinnamon, and mint from middle to elderly age groups.

Table 13. Summary of Specific Sensitivity Test results in regression model of mean threshold test scores (dependent variable) and overall identification score with age.

Mean Threshold Score		Overall Identification Score		Overall Identification Score * Age Group			
				41-60		61-80	
Dependent Variable	R^2	β	p	β	p	β	p
Composite	0.361	-0.01	0.92	0.49	0.08	0.94	< 0.001***
Onion	0.100	0.02	0.88	0.16	0.62	0.65	0.01*
Banana	0.171	-0.01	0.95	0.59	0.06	0.73	0.003**
Cinnamon	0.146	0.01	0.96	0.34	0.29	0.37	0.13
Mint	0.121	-0.19	0.09	0.63	0.05*	0.78	0.002**
Mushroom	0.164	0.10	0.37	0.07	0.81	0.49	0.05*
Popcorn	0.259	-0.06	0.56	0.42	0.16	0.96	< 0.001***
Smoke	0.238	-0.02	0.83	0.21	0.47	0.87	< 0.001***
Cheese	0.223	-0.02	0.82	0.43	0.16	0.73	0.002**
Rose	0.247	0.08	0.42	0.25	0.41	0.48	0.04*

***** $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

5.5. Discussion

This study is the first use of adapted Sniffin' Sticks to determine olfactory sensitivity by measuring both identification ability and threshold of subjects with a common set of single odourants in an Asian population. The use of single odourants which are key aroma compounds in the tested odours at suprathreshold, such as isoamyl acetate in banana, ensured that perception of the odour was not dependent on the combination of various odourants in order to achieve successful identification, eliminating possible interaction effects such as enhancement and suppression (Frank et al., 2010).

Although the age groups were widened to incorporate two decades instead of one for data analysis as in Chapter 4, observed age-related decrease in identification ability and elevation in threshold sensitivity were similar to the results from Chapter 4. Fall in identification rates was minor from young to middle age groups, but accelerated significantly from middle to elderly age groups. In addition, mean threshold scores of the nine odourants declined uniformly and significantly from young to middle, and middle to elderly age groups.

Present findings showed that while mean threshold scores for all the odourants were slightly elevated for subjects who correctly identified the odourants than those who did not, the differences were not significant for five of the nine odourants, indicating an inconsistent relationship. Similar results were also observed during the preliminary Adapted Specific Sensitivity Test (Section 2.4.3.2), where the ability to identify the 10 odourants did not have a significant impact on the detection thresholds in the sampled population. Thus, the ability to identify an odourant does not seem to have a direct consequence

on detection threshold sensitivity for the odourant, in agreement with findings that proficiency in odour identification and detection measure separate olfactory processes (Larsson, Finkel, & Pedersen, 2000).

Similar to the observations in the National Geographic Survey (Wysocki & Gilbert, 1989), odourant age-response curves of successful identification rates and threshold scores for the 9 odourants did not show a consistent relationship between the two olfactory functions.

More significantly, our results demonstrated the moderating effect of age in the relationship between overall identification scores and composite and individual odourant threshold scores in regression analyses. As expected, low and non-significant regression coefficients for overall identification scores in the model indicated weak correlation between overall identification proficiency and threshold sensitivity of the odourants across all age groups. However, regression coefficients of the interaction terms suggest that the overall identification proficiency has an increasingly positive relationship with olfactory sensitivity as one ages, i.e., one's ability to detect certain odours at low concentrations is increasingly related to his or her ability to identify odours. In addition, the influence of age was not uniform across all odourants and differed in rate and extent of moderation.

Savic (2002) found that smelling of familiar odours also elicited the activity of semantic pathways in the brain, as opposed to unfamiliar odours which only activated core olfactory regions. The positive relationship between general semantic knowledge and odour identification proficiency suggest that higher occurrence of odour exposure and odour-name associated knowledge, such as in learning and training, will increase the rate of successful

identification (Hedner, Larsson, Arnold, Zucco, & Hummel, 2010; Larsson et al., 2000; Larsson, Nilsson, Olofsson, & Nordin, 2004). As a consequence, it is then no surprise that there is a growing body of clinical evidence that olfactory training, which involves daily exposure to known odours at suprathreshold for weeks to months, for patients with olfactory dysfunction has a beneficial effect on their olfactory abilities. In addition to increased identification of the odourants used in training kits, olfactory training also improved overall identification proficiency and olfactory sensitivity for other odourants, as evidenced by higher aggregate olfactory test scores (Haehner et al., 2013; Hummel et al., 2007, Konstantinidis, Tsakiropoulou, Bekiaridou, Kazantzidou, & Constantinidis, 2013). Although olfactory training has achieved considerable olfactory function improvement in patients with olfactory loss from Parkinson's disease (Haehner et al., 2013)' infections to the upper respiratory tract, trauma, and idiopathic causes (Hummel et al., 2007; Konstantinidis et al., 2013), it has not been used for subjects with olfactory loss with normal ageing.

A wide range of studies have reported on the decline of olfactory function with age, but the cause of loss remains unknown. Researchers have attributed age-associated modifications in olfactory abilities to increased oxidative stress on the olfactory epithelium and/or decreased regeneration of receptor cells (Ahmed & Haboubi, 2010), cumulative damage to olfactory receptors (ORs) through life and neurological degeneration (Doty, 2009; Wong, Muller, Kuwabara, Studenski, & Bohnen, 2010). In addition, contrary to an earlier study (Paik, Lehman, Seiden, Duncan, & Smith, 1992), more recent findings suggest that while the number of olfactory sensory neurons

(OSNs) did not differ between the young and elderly, there was loss of OSN odour selectivity in the elderly, possibly the result of age-associated dysregulation in the expression of ORs (Rawson et al., 2012).

Present findings support the use of olfactory training to improve age-associated loss of olfactory functions. Frequent exposure, learning, and training to odours, such as in the case of professional perfumers, has shown the ability to counteract age-associated decrease in brain gray matter volume in the central system (Delon-Martin, Plailly, Fonlupt, Veyrac, & Royet, 2013; Montembeault et al., 2012), which in turn is positively correlated to semantic memory. Therefore, olfactory training may negate losses in identification ability and consequently, threshold sensitivity with age. Middle-aged and elderly subjects who regularly experience different odours and/or pay particular interest in discerning between them, such as in cooking and wine tasting, could thus attain higher odour identification proficiency and lowered detection thresholds than their counterparts.

It is also worthy to note that olfactory training may only improve olfactory function for age groups which have experienced substantial age-associated losses, such as the middle to elderly age groups in this study. Our regression analyses support research findings that frequent odour exposure may not induce increase olfactory sensitivity of humans that already possess appreciable sensitivity (Wang, Chen, & Jacob, 2004; Wang, Wysocki, & Gold, 1993), such as that shown in the young to middle-aged groups of our study, where the overall identification proficiency has little relationship with threshold sensitivity.

Although reports showed a general increase in olfactory functions across various odourants with olfactory training, results from the Specific Sensitivity Test indicate that some odourants may be less sensitive to the effect of age moderation, with low interaction regression coefficients between overall identification and mean threshold scores throughout life, such as in the case of cinnamaldehyde. Studies on sensitivity induction of androsterone and isovaleric acid in humans and mice, and diacetyl and linalool of wine tasting experts demonstrated a preferential increase in sensitivity only for the induction odourant (Tempere et al., 2011; Wang et al., 1993; Wang et al., 2004).

The successful olfactory sensitization of humans by repeated exposure of a set of intense odours indicates the existence of plasticity in the human olfaction system, which has only been previously demonstrated in animals (Wang et al., 1993; Youngentob & Kent, 1995). However, the precise mechanism by which olfactory training boosts olfactory abilities has not yet been elucidated. Peripheral plasticity was demonstrated by Wang and group (2004) by increasing electrophysiological (EOG) responses of the olfactory muscosa, where the amplitude is directly proportional to detection threshold sensitivity, for androstenone after repeated exposure of the compound. In addition, in neonatal mice, enhanced odour exposure can increase the number of OSNs which last at least until young adulthood (Rosselli-Austin & Williams, 1990), and in adult mice, on top of an increase in OSNs, larger glomeruli and mucosal activity in the olfactory epithelium also accompanied olfactory learning (Youngentob & Kent, 1995; Yee & Wysocki, 2001; Jones, Choi, Davis, & Ressler, 2008). Selective expression of ORs in response to

odour exposure has also been observed in cell line studies (Keller, Zhuang, Chi, Vosshall, & Matsunami, 2007), which may improve the loss of OSN selectivity as a result of ageing (Rawson et al., 2012). Altogether, sensitization to a wide range of odours via repeated exposure to a subset of odourants, such as in olfactory training, may be the single or cumulative result of various alterations along the olfactory system.

Although results from the Specific Sensitivity threshold subtest differed from findings of Sinding et al. (2014) on the relationship between molecular weight of odourants and detection thresholds with age (Section 4.5.2), the size of odourants in the test had a bearing on the extent of the effect of age on the two measures of olfactory function. Age had the most significant influence throughout adulthood on the relationship between identification proficiency and threshold sensitivity for odourants with the lowest molecular weights [butyric acid (cheese, $M_r = 88.1$), 2-methyl-3-tetrahydrofuranthiol (onion, $M_r = 118.2$), phenylethyl alcohol (rose, $M_r = 122.2$), and acetyl pyrazine (popcorn, $M_r = 122.1$)] of the nine odourants.

Assuming the steric theory of molecules (Amoore, 1964), such that each olfactory receptor (OR) is contoured to complement the shape of one or more molecules and that the affinity between ligand and OR is dependent on how well they fit together, smaller molecules would be able to fit into olfactory binding sites more easily than larger molecules (Sinding et al., 2014). Thus, it is hypothesized here that the selective expression of ORs in response to increased exposure and learning (Keller et al., 2007) aids in overcoming age-associated olfactory loss of low molecular weight odourants in preference

of high molecular weight odourants, which may require higher specificity in the OR binding sites or a larger number of ORs in order to activate a response.

Contrary to many previous studies (Corwin, Loury, & Gilbert, 1995; Doty, Shaman & Applebaum, 1984; Doty, Shaman & Dann, 1984; Katotomichelakis et al. 2007), we did not find gender difference and gender-age interaction for both identification proficiency and threshold sensitivity. Female superiority observed in reports has been attributed to differences in verbal skills, hormones, anatomy, and physiology (Doty, Applebaum, Zusho, & Settle, 1985; Larsson et al. 2004), which may not apply to the subject population in Singapore. While most studies on olfactory function are conducted in Europe and the United States, Singapore is characterized by year-long tropical climate. Considering possible effects of environmental conditions, cultural, language, and dietary differences, data from this study may vary from that of other settings.

To summarize, findings from this study suggest that there is no reliable relationship between the two olfactory functions, identification ability and threshold sensitivity, for a single odourant across all age groups. However, age moderates the relationship between overall identification proficiency and threshold sensitivity of tested odourants in the Specific Sensitivity Test. Moderation was especially pronounced for lower molecular weight molecules, and the results suggest that olfactory training may decrease the rate or compensate for the effects of olfactory loss in the elderly.

CHAPTER 6: INCREASING BEVERAGE PALATABILITY FOR THE ELDERLY BY RATIONAL FLAVOUR MODIFICATION

6.1. Introduction

A census released in June 2014 showed 17.1 % of Singapore's resident population was 60 and over, of which 83.0 % were ethnic Chinese (Department of Statistics, 2014). The proportion of Singaporeans aged 60 and above is projected to rise to 38 % by year 2050 (Singapore Business Review, 2013), an increase from about 600,000 to 1 million elderly citizens in the next 30 years. With a rising population of the elderly, geriatric needs for healthy ageing concurrently increase, including catering to special dietary requirements and preferences of the elderly. Meeting proper nutrition requirements are especially important in chronic disease management and for the elderly who are malnourished.

As mentioned previously, age-related chemosensory losses can affect food intake and variety by altering flavour perception, possibly leading to lower hedonics and food appreciation among the elderly (Donini et al., 2003). There is a lack of literature correlating chemosensory losses and flavour enhancement methods amongst the elderly in Asia as compared to Europe (Koskinen et al., 2003a; Koskinen et al., 2003b; Kremer et al., 2007a; Schiffman & Warwick, 1993). Most studies utilize flavour enhancement methods by increasing the concentration of a flavour in food applications (Koskinen et al., 2003a; Schiffman & Warwick, 1993; Seo & Hummel, 2009).

To address the differential rates of olfactory decline, the flavour modification method was applied in flavours for consumer sensory evaluation. The target flavour, which consists of a number of odourants, was first categorised into odour groups. The concentration of each odour group was either kept constant or adjusted based on findings from the Specific Sensitivity Test (Chapter 4). However, known odour-impact compounds of the target flavours would not be grouped with any other odourants to prevent the odour-impact compounds from affecting the overall flavour and undermine any flavour changes due to alterations in concentrations of the other odour groups.

To evaluate if flavour modification may improve palatability of foods for the elderly, the flavours have to be applied into a medium which minimises influence from other sensory modules, such as touch and sound. The use of beverages minimises the influence of tactile-flavour interactions which may influence the overall flavour perception. Although taste-odourant interactions may vary for different concentrations of odourants applied into the beverages, the resulting changes in overall flavour perception can be attributed solely to

the change in concentration of the odourants by keeping taste-contributing ingredients of the beverage applications constant. Consumer sensory evaluation was carried out on two age groups, the young (21-35 years old) and the elderly (61-75 years old (WHO, 2013)), to investigate if flavour modification is an effective technique in the compensation of elderly olfactory loss as compared to the flavour enhancement method.

6.2. Aims and Objectives

This study aimed to explore differences in preferences and perceived intensities of young and elderly Chinese Singaporeans and Singapore Permanent Residents (PRs) towards flavours prepared using the flavour modification and enhancement methods.

6.3. Materials and Methods

6.3.1. Preliminary Survey

The preliminary flavour survey results were collated from 205 Chinese Singaporeans/Permanent Residents (PRs) aged 51 and above (Table 14; mean age = 59.8 ± 7.44; range from 51 – 87 years old). Respondents were asked to select their top 5 favourite flavours (not in any particular order) from a list of 25 common flavours in order to determine which flavours were to be applied. Of the 205 respondents, 58 were done in person while the rest was carried out via the emails and social media. Respondents could choose to do the survey either in English or Mandarin (Appendices 2 and 3).

Table 14. Demographic information of preliminary flavour survey respondents.

Age Group	Gender	
	Female	Male
51-60	89	42
61-70	30	25
71-80	7	9
81-90	2	1
Total	128	77

Coffee and mango were the top two flavours of the 25. Among the target consumer group for the consumer sensory evaluation (61 – 75 years old, n = 59; 28 males, 31 females; mean age = 66.7 ± 4.38 years), respondents liked coffee the most (30 votes), after which was almond (19 votes), peach (18 votes), orange (18 votes), and mango (17 votes). Coffee and mango were selected for modification and application due to the relatively high exposure of both the young and elderly groups in Singapore to these natural products and versions of the coffee- and mango- flavoured processed products. In addition, coffee and mango flavours present the opportunity for different beverage applications, namely mango-flavoured beverage and instant coffee, and will hence provide a broader insight to the flavour modification method.

6.3.2. Flavour Modification and Application

Flavour modifications were achieved by adjusting specific odour groups based on results of the Specific Sensitivity Test, while keeping the remaining odour groups constant.

Separation of odourants into odour groups was first based on databases of flavour and fragrance odourants (Leffingwell & Associates, n.d.; LRI &

odour database, n.d.; Taytonn, n.d.; The Good Scents Company [tgsc], n.d.) then confirmed by evaluating each individual compounds through sniffs using sniffing strips dipped with the odourant and directly from the bottle. Currently, there is no universally accepted comprehensive system of odor classification although groups of odors by perceptual similarity exist, even across cultural boundaries (Auffarth, 2013), thus this method of grouping was chosen for the purpose of this study.

The flavour carriers triacetin (TA) and propylene glycol (PG) were used for the mango formulation while only propylene glycol was used for the coffee formulation. Odourants of each odour group (with the exception of the "fruity" group in the mango formulation) were mixed into PG such that odourants made up 50 % w/w in each odour group to increase the stability of the mixtures by reducing evaporation rates of the volatiles and preventing growth of bacteria and fungi (De Spiegeleer et al., 2006). The "fruity" odour group in the mango flavour was made up of 30 % w/w TA, 20 % w/w PG instead of 50 % w/w PG to ensure complete miscibility of odourants. The odour groups were freshly prepared monthly to prevent volatilisation or interaction of the compounds which would result in less-than-expected amounts of compounds being added during flavour modification. The amount of each odourant added in the respective odour group was based on a commercial formulation provided by KH Roberts Company.

Four versions of each flavour were prepared using the odour group mixtures: 1) Original, 2) Modified Version 1, 3) Modified Version 2, and 4) Enhanced. The original flavour was made up with the same ratios of odourants as in the original formulation. Flavour modification was made by combining

the odour groups in ratios as shown in (Tables 15 and 16). The “sulfurous” group was unadjusted for both flavours as its representative odourant in the Specific Sensitivity Test, 2-methyl-3-tetrahydrofuranthiol (onion), decreased the least with age, remaining almost constant. Thus, the sulphurous group and changes in threshold of 2-methyl-3-tetrahydrofuranthiol was used as a baseline to determine the necessary adjustments to the concentrations of the other relevant odour groups. The adjustments were made based on the extents of olfactory losses as determined before. The fourth version, Enhanced, was applied using the flavour enhancement method. An increase in flavour dosage of the original version was made during application to beverages to simulate that of the enhanced version. To determine the increase in flavour dosage of the original version, the top note of the highest adjusted odour group in version 2 (“floral” for mango and “roasted, nutty” for coffee) was matched with that of the enhanced versions in the application solution (Table 19). Thus, a total of 4 samples for each flavour were chosen after preliminary testing. The final flavour dosages in the respective application solutions are shown in Table 19.

Table 15. Odour group composition of three versions of mango flavour, original and modified versions 1 and 2.

	Odour Group	Amount added in original version (%)	Ratio of change with respect to sulfurous odour group	Version 1		Version 2	
				No. of times increase (X)	Amount added (%)	No. of times increase (X)	Amount added (%)
Adjusted	Citrus	0.09	3.89	1.33	0.12	1.56	0.14
	Floral	0.13	35.9	4.00	0.52	6.00	0.78
	Fruity	0.50	2.78	1.24	0.62	1.38	0.69
	Sour	0.72	4.22	1.35	0.97	1.58	1.14
Unadjusted	Sulfurous	1.52	1.00		1.52		1.52
	Caramellic	11.30			11.30		11.30
	Sweet, creamy	0.14			0.14		0.14
	Vegetable	0.06			0.06		0.06
	Fruity, minty	0.06			0.06		0.06
	Green	0.38			0.38		0.38
Carriers	Propylene glycol	84.13			83.38		82.880
	Triacetin	0.97			0.93		0.90
	Total	100.00			100.00		100.00

Table 16. Odour group composition of three versions of coffee flavour, original and modified versions 1 and 2.

	Odour Group	Amount added in original version (%)	Ratio of change with respect to sulfurous odour group	Version 1		Version 2	
				No. of times increase (X)	Amount added (%)	No. of times increase (X)	Amount added (%)
Adjusted	Fruity	0.15	2.78	3.00	0.45	3.60	0.54
	Sour	0.05	4.22	4.00	0.20	5.00	0.25
	Roasted, nutty	0.55	4.11	3.93	2.16	4.91	2.70
Unadjusted	Sulfurous	0.08	1.00		0.08		0.08
	Sweet, creamy	0.40			0.40		0.40
	Caramellic	9.94			9.94		9.94
	Buttery	1.40			1.40		1.40
Carrie	Propylene glycol	87.44			85.37		84.69
	Total	100.00			100.00		100.00

Beverage applications of the flavours were based on basic commercial formulations for flavoured sugar beverages and instant coffee 3-in-1 (Table 17 and Table 18).

Table 17. Base composition for beverage application of mango flavour.

Ingredients	Percentage by weight of compound/volume of water (% w/v)
Castor sugar	8.000
Malic acid	0.030
Tartrazine	0.006
Water	91.964
Total	100.00

Table 18. Base composition for instant coffee 3-in-1 for the application of coffee flavour.

	Ingredients	Percentage by weight of compound/volume of water (% w/v)
Premix	Castor sugar	8.00
	Non-dairy creamer	4.50
	Instant coffee	1.30
	Salt	0.03
	Water	86.17
	Total	100.00

For consumers' sensory evaluation, all mango flavours were dosed into the application solution at room temperature within 2 hours from commencement of the starts of the test. The coffee application solution was kept in a water bath of 70 ± 3 °C. Dosages were as described in Table 19. During the break between sensory evaluations of the two flavours by consumers, the respective coffee flavours were dosed to minimise vaporisation and losses of the flavours due to the elevated temperatures.

Table 19. Flavour dosages of the original, modified (1 and 2) and enhanced versions in mango and coffee beverage applications.

Version	Percentage by volume of flavour dosage/volume of application solution (% v/v)	
	Mango flavour	Coffee flavour
Original and modified versions (Versions 1 and 2)	0.175	0.15
Enhanced version	1.05[1]	0.75[2]

[1]Flavour dosage of enhanced = 0.175 X 6 (since there is a 6X increase in floral note; Table 15).
[2]Flavour dosage of enhanced = 0.15 X 5 (since there is a 4X increase in sour note; Table 16).

6.3.3. Consumer Sensory Evaluation

The consumer sensory evaluation test was approved by Institutional Study Board (IRB) of the National University of Singapore (IRB reference code: 13-360E).

All questionnaires, briefings, and evaluations were translated from English to Mandarin for subjects who preferred using Mandarin. The screening questionnaire was done to ensure recruited subjects met the eligibility criteria. Subjects were recruited via the university, online social media, community centres, and elderly day care centres.

6.3.3.1. Participant Screening and Demographics

Subjects were first screened for the following criteria via online forms: 1) citizenship or permanent residence in Singapore, 2) free from food allergies, 3) in general good health and must not have had losses in their sense of smell due to illnesses, head injuries, or diagnosed anosmia/hyposmia, and 4)

familiarity with mango fruit or mango-flavoured products, i.e. consumed at least once in the past 3 months, and coffee, i.e. consumed at least twice a week, so as to reduce random judgments due to unfamiliarity.

Another consideration during the screening of subjects was whether they had done any similar food research studies recently (within the past one year). The internal representations and expectations of consumers who have done sensory evaluation recently (in the past 1 year), especially on products similar to the current samples in this research, may differ from those who have not (Lawless and Heymann, 2010). As such, the preference and intensity ratings are likely to be affected for those who have done sensory evaluation recently. However, few such subjects were encountered in the current research and hence did not pose as a problem during recruitment of subjects.

One hundred and twenty subjects were recruited (Table 20) for consumer sensory evaluation of the mango and coffee flavours in beverage applications. To prevent the subjects' sense of smell from being affected and/or impair their judgment during the test, they were advised against: 1) food and beverage consumption apart from water (especially strong-smelling foods such as durian, coffee, garlic and onion) at least half an hour from the test, 2) wearing scented products, and 3) smoking before and/or during the test. Subjects were also advised to reschedule if feeling unwell or were ill. Secondary forms were completed by subjects before the start of the test to obtain information on: 1) food and beverage consumption apart from water, 2) confirmation that scented products were not applied before the test, and 3) the last time of consuming mango fruit/flavoured product(s) and coffee.

Table 20. Demographic information on recruited subjects for the consumer sensory evaluation test.

Age group	Males	Females	Total
21 – 25	9	12	21
26 – 30	14	11	25
31 – 35	9	5	14
Total	32	28	60
61 – 65	7	19	26
66 – 70	7	11	18
71 – 75	4	12	16
Total	18	42	60

6.3.3.2. Sensory Evaluation Test Procedures

Prior to the sensory evaluation, subjects were briefed on the objectives and procedures of the consumer sensory evaluation. Subjects signed off on participation information sheets and consent forms before commencement of the test.

Sensory evaluation was performed in the sensory laboratory of the Food Science & Technology Programme at the National University of Singapore. The sensory laboratory is equipped with individual booths and FIZZ sensory system (FIZZ, Biosystems, Couternon, France) for the tests to be conducted and integrated using computer software. However, for Mandarin-speaking and reading subjects, forms were translated and prepared for the subjects to be completed by hand. Subjects were seated in individual booths in the sensory laboratory. Each sample was labelled with a 3-digit code and 40 mL for each sample was given in a 60 mL plastic sample cup.

Sensory evaluation procedures for both flavours were exactly the same but divided into separate parts for each flavour evaluation. The 4 versions of

each flavour are evaluated firstly on (1) preference (how much they liked/disliked a sample), followed by (2) overall intensity (overall intensity of the mango/coffee flavour). Subjects were advised to drink as much of the sample as they needed to form a response about each sample. Instructions were given during the briefing and throughout the test for the subjects to drink filtered water and/or consume unflavoured Water and Carr's table water crackers (United Biscuits, UK) for palate cleansing during the test. Due to the long-lasting after effects of coffee and coffee flavours (Seo, Lee, & Hwang, 2009), mango-flavoured beverage evaluation was conducted before coffee. Between evaluations of the two applications, a 10 minute break was given for every subject to drink water and consume the crackers in the booth in order to minimize carryover of the mango flavour into the coffee evaluation.

All the four samples (original, versions 1, 2 and enhanced) were presented on the same page per section to prevent subjects from avoiding usage of the scales' ends in expectation of a stronger/weaker or more liked/disliked sample later on in the evaluation (Lim, 2011). Each preference section also included an open-ended question (asking what the subjects liked/disliked about the samples) to verify reasons for their preference. The last question in each flavour evaluation was optional for subjects to provide any other comments.

Since the main goal of the evaluation was to determine how much subjects from the two different age groups liked a particular sample, scales using in affective testing were considered: (1) structured, (2) unstructured (ULS) and (3) hybrid scales which were conceived based on ULS (Lim, 2011). Preliminary trials with small groups of subjects using the hybrid and

structured scales demonstrated that subjects were more discriminating when using the hybrid scale, which may be due to the limited freedom of subjects to express the full range of hedonic experiences due to limited response categories when using the 9-point scale (Lim, 2011). However, as the hybrid scales have only been utilised in a few studies (Da Silva et al., 2013; Villanueva et al., 2005; Villanueva & Da Silva, 2009), the unstructured line scale (ULS) was preferred. As compared to the structured 9-point scale, the ULS was more sensitive and offered subjects greater freedom to express sensory perceptions (Da Silva et al., 2013). Moreover, the ULS also had decreased contextual effects favouring its use in intercultural studies, thereby minimising effects due to different interpretations of scale anchors in different languages (Da Silva et al., 2013). The ULS used in the sensory evaluation test was anchored at the ends with the verbal terms extremely disliked/weak and extremely liked/strong with regards to preference and overall flavour intensities, respectively (Kremer et al., 2007a; Kremer et al., 2007b; Seo & Hummel, 2009). The length of the ULS was proportional to that of a 10-point scale such that 1 = extremely disliked/weak and 10 = extremely liked/strong.

6.3.4. Statistical Analysis

Consumer data was analysed using the SPSS V22.0 (SPSS Inc. Chicago, IL, USA). Prior to data analysis, the raw data was checked for normality for preference/intensity ratings by age group: 21 – 35 (termed as "young") and 61 – 75 (termed as "elderly"). One-way ANOVA was done on preference ratings for both flavours using age group (2) as the factor.

Two-way ANOVA was carried out for age group and gender with preference ratings to investigate interaction effects. Pearson's correlation test was used to evaluate the impact of perceived intensities on preference ratings. Differences in perceived intensities was tested using one-way ANOVA and was conducted separately for the age groups and flavours as factors.

To evaluate the effect of preference for the original flavour, each age group was divided into "low preference" (original preference ratings < median) and "high preference" (original preference ratings ≥ median). The data was analysed with two-factor and one-way analysis of variance (ANOVA) and post-hoc Tukey HSD tests.

Regression analyses were conducted to evaluate the influence of the follow variables on the preference and/or intensity ratings: time of last mango fruit/flavoured product(s)' consumption (2; within the week/more than a week or unable to specify date, 2; within the month/more than a month or unable to specify date), time of last coffee consumption (2; whether or not coffee was consumed on the test day/the day before), illnesses/long-term medication known to affect sense of smell (2; none/have been diagnosed and/or on medication), as well as odourants worn by subjects (2; not wearing/wearing).

6.4. Results

Sensory evaluation of the four versions of each flavour, mango and coffee took 30 to 45 minutes for each participant.

6.4.1. Preference Ratings for Mango and Coffee Flavour Samples

The mean preference ratings of all recruited participants for the four mango flavour versions are presented as a function of age groups in Figure 19. Mean preference ratings for young (21-35 years old, n = 60) and elderly (61-75 years old, n = 60) for 4 coffee samples (original, version 1, version 2, and enhanced)..

Contrary to expected findings, the young consumers preferred version 1 the most (5.95 ± 1.81) while the elderly preferred the original mango flavour the most (6.20 ± 2.48). Both young (4.22 ± 1.84) and elderly (4.93 ± 2.78) subjects least preferred the enhanced sample (Figure 18).

Similar findings were found for the four coffee flavour versions (Figure 19). The young preferred version 1 the most [6.07 ± 2.15] while the elderly preferred the original the most [6.26 ± 2.42]. Both the young [2.46 ± 1.14] and the elderly [5.44 ± 2.85] least preferred the coffee enhanced sample (Figure 19).

Preference ratings for original and modified samples of both mango and coffee flavours among the young and elderly groups were above 5, indicating a general liking for the flavours applied into beverages. Enhanced mango and coffee flavours had below 5 preference ratings, except for enhanced coffee preference ratings by elderly subjects (Figure 19). There was

no significant difference found for mean preference ratings of all samples for both flavours between age groups ($p \geq 0.05$), except preference ratings for coffee original [$F(1,118) = 5.20$, $p = 0.02$] and coffee enhanced [$F(1,108) = 48.31$, $p < 0.001$]. The young rated the original version for coffee lower [5.28 ± 2.31] than the elderly [6.26 ± 2.42]. The enhanced version for coffee was rated significantly lower by the young age group [2.46 ± 1.14] as compared to the elderly [5.44 ± 2.85].

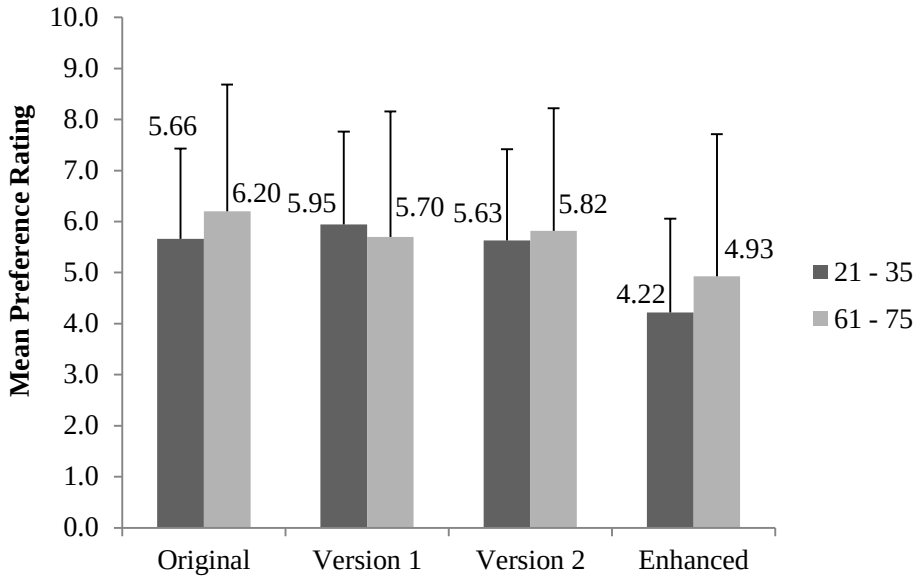


Figure 18. Mean preference ratings for young (21-35 years old, *n* = 60) and elderly (61-75 years old, *n* = 60) for 4 mango samples (original, version 1, version 2, and enhanced).

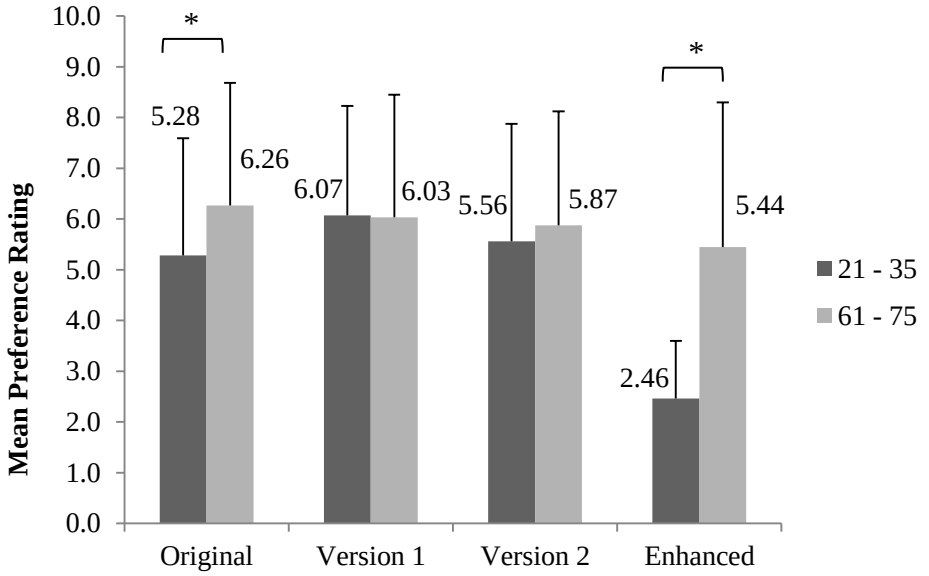


Figure 19. Mean preference ratings for young (21-35 years old, *n* = 60) and elderly (61-75 years old, *n* = 60) for 4 coffee samples (original, version 1, version 2, and enhanced).
* Denotes significant difference between mean threshold scores by one-way ANOVA.

There was no significant interaction effect found between age and gender for all mean preference ratings ($p \geq 0.05$), with the exception of mango enhanced [$F(1,3) = 6.72$, $p = 0.01$]. There was no significant difference in the mango enhanced preference ratings among the young and elderly females [$F(1, 62) = 0.001$, $p = 0.98$] but elderly males rated preference for the mango enhanced version significantly higher (6.43 ± 1.93) than that for the young males (4.08 ± 1.56) [$F(1,45) = 20.78$, $p < 0.001$]. Mean preference ratings for mango enhanced version did not significantly differ between genders for the young [$F(1,54) = 0.63$, $p = 0.43$] but there was a significant difference found for the elderly [$F(1,53) = 6.31$, $p = 0.02$]. Elderly males were found to rate the preference for the mango enhanced version higher (mean preference rating = 6.43 ± 1.93) than that of elderly females (mean preference rating = 4.46 ± 2.96).

Illnesses and long-term medication known to affect sense of smell and scented products were not found to affect preference and/or intensity ratings and hence, these results were not included.

6.4.2. Preference Ratings as a Function of Liking for Original Flavour

As the original mango and coffee flavours applied were not evaluated for consumer preference before sensory evaluation tests, results obtained may be a reflection of liking for the original flavour. To evaluate how the preference ratings for modified and enhanced versions of mango and coffee flavours, each age group was further divided into 2 subgroups: 1) subjects who rated the original mango flavour preference rating below median preference

rating for the original flavour (Mango: original rating < 5.55 for the young, original rating < 6.47 for the elderly; Coffee: original rating < 5.28 for the young, original rating < 6.49 for the elderly), termed "low preference", and 2) subjects who rated the original mango flavour at and above the median score, termed "high preference".

For both the young and elderly subjects, low preference individuals preferred modified versions of mango and coffee flavours, whereas high preference individuals preferred the original flavours over the rest of the samples (Figures 20-23).

Looking first at the mango samples, among the young subjects, there were significant differences in mean preference ratings between the samples for both low [$F(3,116) = 4.00$, $p = 0.01$] and high [$F(3,116) = 17.7$, $p < 0.001$] preference groups (Figure 20). In the low preference group, modified version 1 of mango had significantly higher preference ratings than original and enhanced samples, while in the high preference group, the enhanced mango sample was significantly lower than the rest. Among the elderly subjects in the low preference group [$F(3,116) = 3.17$, $p = 0.03$], preference for modified version 2 sample was significantly higher than enhanced, and in the high preference group [$F(3,116) = 5.32$, $p = 0.002$], original flavour had significantly higher preference to the rest of the samples (Figure 21).

Findings for coffee samples were of the same trend. Young subjects with low preference for the original flavour significantly preferred version 1 over original and enhanced samples, while version 2 was higher than enhanced [$F(3,116) = 11.3$, $p < 0.001$] (Figure 22). Young subjects with high preference for the original flavour rated the enhanced coffee significantly less than the

rest of the samples [$F(3,116) = 22.9$, $p < 0.001$]. Although no significant differences were found between the elderly subjects in the low preference group [$F(3,116) = 2.36$, $p = 0.08$], high preference subjects rated the original coffee flavour significantly higher than the rest of the samples [$F(3,116) = 5.55$, $p = 0.001$] (Figure 23).

Observed results indicate that consumer acceptance for the original flavours had a significant impact on the preference ratings of the modified and enhanced versions by both the young and the elderly.

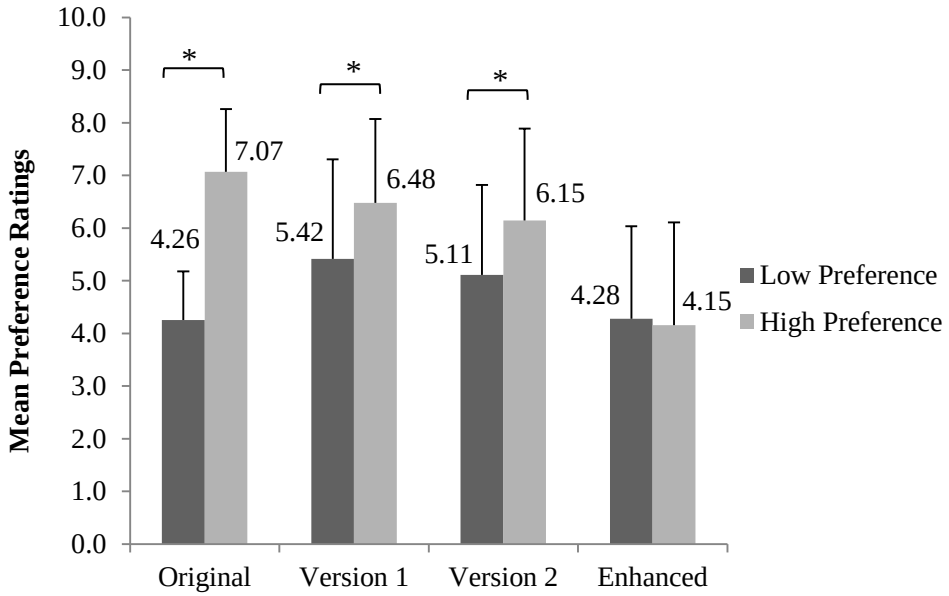


Figure 20. Mean preference ratings of young subjects for 4 mango flavour samples (original, version 1, version 2, and enhanced) as a function of low (*n* = 30) and high (*n* = 30) preference for the original mango flavour.
* Denotes significant difference between mean threshold scores by one-way ANOVA.

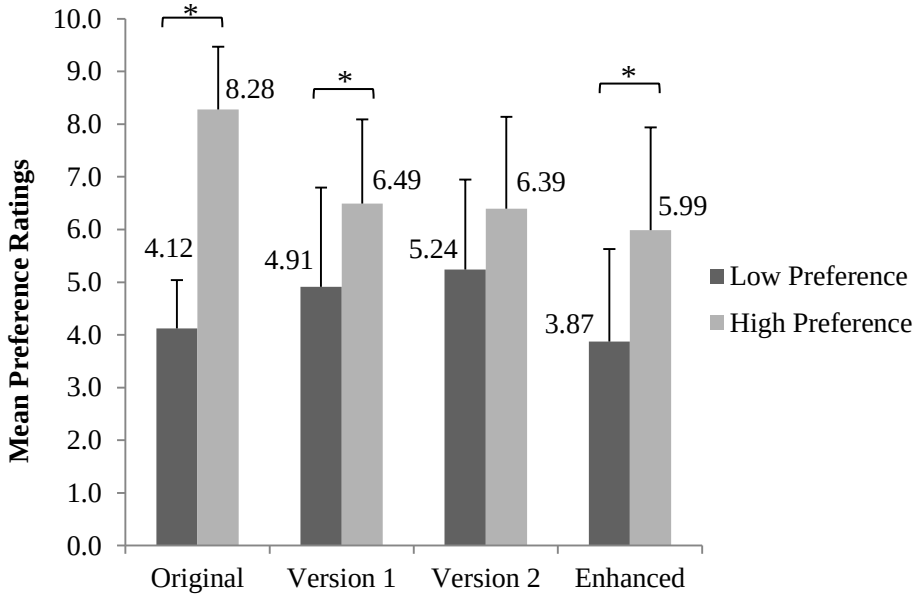


Figure 21. Mean preference ratings of elderly subjects for 4 mango flavour samples (original, version 1, version 2, and enhanced) as a function of low (*n* = 30) and high (*n* = 30) preference for the original mango flavour.
* Denotes significant difference between mean threshold scores by one-way ANOVA.

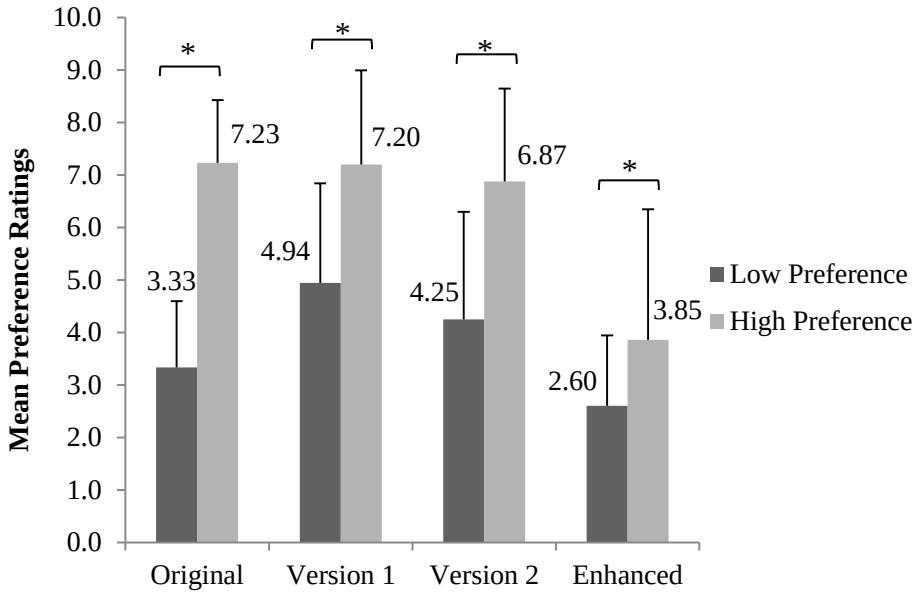


Figure 22. Mean preference ratings of young subjects for 4 coffee flavour samples (original, version 1, version 2, and enhanced) as a function of low (*n* = 30) and high (*n* = 30) preference for the original coffee flavour.
* Denotes significant difference between mean threshold scores by one-way ANOVA.

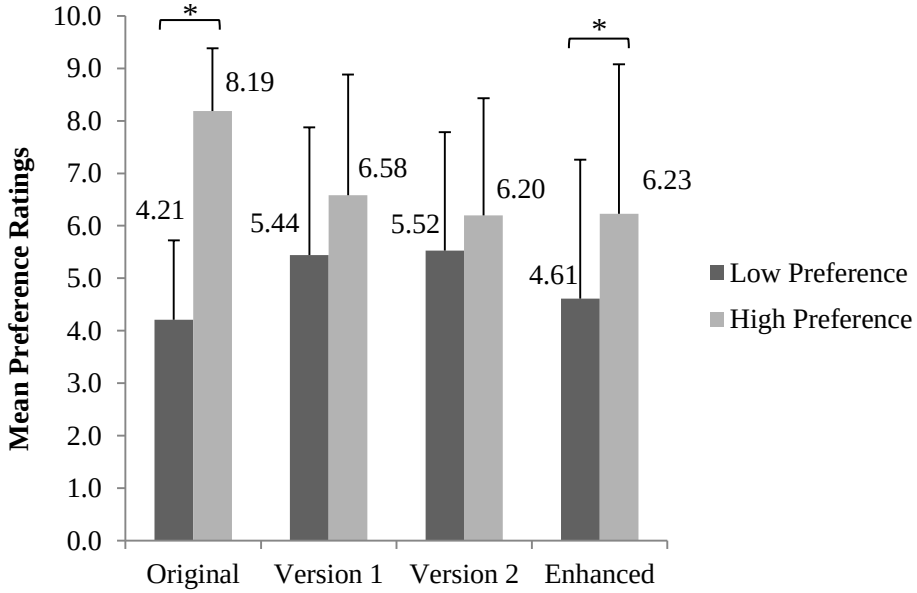


Figure 23. Mean preference ratings of elderly subjects for 4 coffee flavour samples (original, version 1, version 2, and enhanced) as a function of low (*n* = 29) and high (*n* = 31) preference for the original coffee flavour.
* Denotes significant difference between mean threshold scores by one-way ANOVA.

6.4.3. Perceived Intensities and Preference Ratings

The differences in mean intensity ratings for the samples were analysed separately for the young and elderly. For the young, mean intensity ratings were found to be significantly different for mango [$F(3,236) = 4.42$, $p = 0.005$] but not for coffee [$F(3,236) = 0.40$, $p = 0.76$]. Post-hoc Tukey's HSD tests indicated that the mean score for the enhanced version [6.46 ± 2.34] was significantly higher from the original [5.31 ± 2.13], version 1 [5.33 ± 1.70], and version 2 [5.45 ± 1.87] for the young ($p < 0.05$). For the elderly, mean intensity ratings were not found to be significantly different for the mango [$F(3,236) = 0.71$, $p = 0.55$] and coffee flavours [$F(3, 236) = 0.11$, $p = 0.96$].

To investigate if intensity ratings had an effect on preference ratings, the ratings were tested for correlation. Pearson's coefficients of correlation between perceived intensities and preference ratings of all the flavour samples were significant for the all the subjects ($p < 0.05$).

Among the young, a strong, positive correlation was found between intensity and preference ratings for the original version and version 1 for mango ($r_{60}, > 0.50$, $p < 0.001$) (Table 21). A moderate, positive correlation ($r_{60} = 0.46$, $p < 0.001$) was found between intensity and preference ratings for version 2 of mango. There was a low and non-significant coefficient of correlation between intensity and preference ratings for enhanced version of mango ($r_{60} = 0.06$, $p \geq 0.05$).

A strong, positive correlation was also found for the young subjects between intensity and preference ratings for the versions 1 and 2 for coffee ($r_{56} > 0.5$, $p < 0.05$), and a moderate correlation between intensity and

preference ratings for the original coffee flavour ($r_{60} = 0.62$, $p < 0.001$) (Table 22). The coefficient of correlation between intensity and preference ratings for the enhanced coffee flavour was low and not significant ($r_{49} = 0.19$, $p = 0.14$).

Among the elderly, strong, positive correlations were found between intensity and preference ratings for all samples ($r_{60} > 0.5$, $p < 0.001$), except the original version for mango. There was a moderate and positive correlation ($r_{60} = 0.44$, $p < 0.001$] found between intensity and preference ratings for original version of mango.

Table 21. Pearson's coefficients of correlations between perceived intensities and preference ratings among the young.

Flavour	Sample	Pearson's Coefficient of Correlation, *r*	*p*
Mango	Original	0.72	< 0.001**
	Version 1	0.54	< 0.001**
	Version 2	0.46	< 0.001**
	Enhanced	0.06	0.68
Coffee	Original	0.45	< 0.001**
	Version 1	0.62	< 0.001**
	Version 2	0.62	< 0.001**
	Enhanced	0.19	0.14

**difference is significant at the 0.01 level

Table 22. Pearson's coefficients of correlations between perceived intensities and preference ratings among the elderly.

Flavour	Sample	Pearson's Coefficient of Correlation, *r*	*p*
Mango	Original	0.44	< 0.001**
	Version 1	0.56	< 0.001**
	Version 2	0.53	< 0.001**
	Enhanced	0.51	< 0.001**
Coffee	Original	0.53	< 0.001**
	Version 1	0.62	< 0.001**
	Version 2	0.52	< 0.001**
	Enhanced	0.50	< 0.001**

**difference is significant at the 0.01 level

6.4.4. Exposure to Flavour and Preference and Intensity ratings

Last mango fruit/flavoured product(s) consumption was based on: 1) within the week; $n = 43$, and 2) more than a week; $n = 77$. Results were found to be not significant [$p \geq 0.05$], with the exception of mean preference ratings for mango original [$F(1,118) = 4.48$, $p = 0.04$]. Mean preference rating for the original version of mango was higher for those who consumed mango fruit/flavoured product(s) within the week [6.48 ± 2.17] as compared to those who had not [5.63 ± 2.11].

Last coffee consumption was based on: (1) had coffee either on the test day or the day before; $n = 86$ (young = 32, elderly = 54) and (2) did not have coffee either on the test day or the day before; $n = 34$. Both mean preference and intensity ratings for all coffee samples were found to be not significant [$p \geq 0.05$] with respect to coffee consumption.

6.5. Discussion

The present study attempted to address failure of the flavour enhancement method as reported in literature by proposing a flavour modification method to compensate for the age-associated loss in olfactory function of the elderly. However, it was found that the elderly preferred original versions of both coffee and mango flavours, whereas the young preferred version 1 for both flavours.

The flavour modification method process included the isolation of known odour-impact compounds of the flavours, such as ocimene and furaneol which provides characteristic mango-like and sweet notes of mango

(Munafo et al., 2014), respectively, and furfuryl mercaptan which gives the roasted note characteristic of coffee (Blank, Sen, & Grosch, 1991). As such, with increased concentrations of other odour groups, distinguishing notes of the flavours may have been inevitably reduced in comparison to more floral, fruity, and sour notes of the modified mango flavours, and fruity, sour, and nutty notes of the modified coffee flavours.

Of the subjects who were able to recall the last mango or mango-flavoured product consumed within a week of the day of the test, 10 out of 22 of the young subjects had consumed dried mango, mango fruit, or mango juice, whereas 21 out of 28 of the elderly subjects quoted having consumed dried mango, mango fruit, or mango juice, whilst the rest had processed mango products, such as pudding, yogurt and sweets. Similarly, 54 of the 60 elderly participants consumed coffee on the day of or day before the sensory test, whereas only 32 of the 60 young participants reported the same.

Zandstra and de Graaf (1998) have observed in their study on sensory perception and perceived pleasantness of orange beverages that intensity ratings of elderly subjects were affected when they found their interpretation of an ‘orange’ flavour did not correspond to the flavour of the real fruit. Here, we conjectured that the gap in concept of the flavours, in this case the flavour of a mango fruit and flavour of brewed coffee, with the flavour mixtures used in this study, also affected hedonic ratings of the elderly. Modified versions of the mango and coffee flavours may not be representative of the original fruit and beverage, thus there was a preference for the original flavour observed in the elderly.

In contrast, the frequent consumption of processed products using added flavours by young subjects may have had an influence on the acceptance of varied and non-representative flavour profiles of mango and coffee. As a result, we observed preference for mango modified flavours with more floral and fruity notes and coffee with nuttier notes by the younger sample population. In addition, lower preference ratings for modified versions of the flavours in the elderly may also be the result of an increased tendency to avoid or be reluctant to try novel foods (neophobic), such as new mango and coffee flavour profiles, as compared to the young (Kremer et al., 2007a; Meiselman et al., 2010).

Nevertheless, similar to findings by Kremer et al. (2007a) in the group's study on cheese flavour enhancement in savoury waffles, the flavour enhancement method was not preferred over the original flavour. In addition, enhanced samples did not perform better than the proposed flavour modification method in this study, as the elderly rated preference for the enhanced version lowest amongst all the samples for both coffee and mango flavours.

Splitting the young and elderly groups by their preference rating for the original flavours, the results revealed that elderly subjects who preferred the original mango and coffee flavours rated those flavours significantly higher than the other versions, while young subjects with high preference for the original flavours did not show statistically higher preference for the original samples. Such an observation is in support of the elderly subjects' preference for flavours which fulfil their concept of flavours with profiles representative of the real fruit or beverage.

The subgroups also brought to light a contribution factor to higher ratings of the modified version 1 in the young group. Although young subjects who liked the original samples also had high preference ratings for the modified versions, subjects who did not like the original flavours had significantly higher preference ratings for modified version 1. In contrast, the difference in ratings by low preference elderly subjects who preferred the modified versions more than the original samples did not reach statistical significance. While further work needs to be done in order to ascertain the trends we observed in the results here, it is apparent that when conducting research for compensation strategies to increase palatability of food for the elderly, it is essential to ensure the flavours and ingredients used are favoured by the subjects.

In comparing intensity ratings for mango and coffee samples between the young and elderly, only a significant difference was detected for mango intensity ratings among the young, while the adjusted odour notes in the modified versions were insufficient to create a perceptual difference in intensity for the elderly. The same finding was observed for the coffee samples among the young.

Mango flavour versions with the strongest odour notes, version 2 and enhanced, were found to differ significantly from the other samples for the young. Although such findings indicate the inability of the elderly to perceive differences in overall flavour of the samples due to diminished olfactory function, it also indicates that a greater intensification of odour notes in the enhanced and modified versions may be necessary to reach the just-right intensities and create a perceptual difference. Moreover, difficulty experienced

by the elderly in reconciling the expected real fruit or coffee flavour with the flavour in the samples they were consuming may have affected the intensity ratings of the flavours in the beverages (Zandstra & de Graaf, 1998).

The ambiguity in studies correlating flavour perception, hedonics and food intake may be due to other factors as well; such as physiological factors and the use of different methodologies. Koskinen et al. (2005) acknowledged that differences in response behaviours in the younger and older age groups such as the difficulties in expressing their dislike and inexperience in using any kind of scale may explain the different hedonic responses. Other considerations in the studies for the older age group was that oral health and dentition could also impede release and retronasal transport of odours from the mouth to the olfactory receptors (Kremer et al., 2007a).

The medical conditions concerning subjects involved in this research (diabetes, hypertension, high cholesterol, flu/common cold/sinus) were also known to affect one's sense of smell; this has been attributed to the illness and/or consumption of drugs (Ackerman & Kasbekar, 1997; Doty et al. 2008; Schiffman, 1997). Due to the complexity and lack of understanding of mechanisms involved in the nature, incidence and prevalence of drug-related chemosensory disturbances, as well as the physiological basis underlying such disturbances, variations in the consumption periods, drug dosage and medical conditions may affect olfactory functions to varying extents (Ackerman & Kasbekar, 1997; Doty et al., 2008). Although these considerations were not investigated in the current research, it was important to consider the possible effect of medical conditions on an individual's sense of smell since the overall flavour representation may be distorted and hence, any changes in preferences

and/or intensity ratings by the elderly as compared to the young would not be solely attributed to age-related olfactory losses.

Odour perception from the consumption of food and beverages is derived from two sources of olfactory stimuli. One of which is orthonasal, sniffing volatiles through the nostrils into the nasal cavity, and the other, retronasal, through the mouth, behind the palate, and into the nasal cavity when air is pushed up from the trachea (Shepherd, 2006). The Specific Sensitivity Test evaluated the orthonasal olfactory sensitivity of subjects with age, which differ from changes in retronasal olfactory sensitivity, due to interactions with oral and physical conditions, including saliva composition, mastication, and swallowing (Bojanowski & Hummel, 2012). In addition, stimuli from orthonasal and retronasal routes activate different neural processes and brain areas (Shepherd, 2006; Small, Gerber, Mak, & Hummel, 2005; Small, Jones-Gotman, Zatorre, Petrides, & Evans, 1997). Thus, for the successful application of flavour modification to improve palatability of food for the elderly, further research in age-associated changes in retronasal olfactory function is necessary.

Future work on retronasal olfactory function testing on a Singapore population should utilize the same odourants used in the Specific Sensitivity Test for the comparison of sensitivity via the two different routes. To avoid gustatory, thermal, and mechanical sensations through the use of solids or liquids in the buccal cavity, a test for retronasal olfactory function has been developed by Heilmann et al. (2002), utilizing grocery-available powders (Rombaux et al., 2006), such as spices and instant drinks. The powders are applied directly to the tongue using squeezable plastic vials with a long spout,

thus minimizing issues involving area of stimulation and differences in cavity size (Heilmann et al., 2002). However, to further reduce the influence of oral stimulation, odourant delivery containers of diluted odourants with a straw inserted into the headspace may be used to provide pure vapour-phase stimuli to subjects (Chen & Halpern, 2008; Stephenson & Halpern, 2009). Retronasal olfactory function tests in a Singapore population will need to be developed and validated before application to investigate retronasal olfactory function changes with age.

In summary, the proposed flavour modification method to compensate for heterogeneous olfactory loss with age was not confirmed to be effective in this present study. Elderly expectations of the mango and coffee flavours to be representative of flavour profiles of the real mango and brewed coffee may have resulted in higher preference for the original flavours over the modified flavours. In addition, this study revealed the need to utilize flavours which are favoured by the subjects for modification or enhancement in research on compensation techniques.

CHAPTER 7: CONCLUSION AND FUTURE WORK

In this research, the Specific Sensitivity Test was adapted, developed, validated, and conducted on a Singaporean population to determine the changes in olfactory functions, specifically odour identification ability and detection threshold sensitivity, of ethnic Chinese Singaporeans and Singapore Permanent Residents with age. Applying the results of the Specific Sensitivity Test in mango and coffee flavours, an alternative technique, termed here as modification, to compensate for olfactory loss of the elderly and increase palatability of food applications, was developed and used in a consumer sensory evaluation with young and elderly subjects.

The adapted Specific Sensitivity Test was validated to be an effective and reproducible method to determine identification ability and threshold sensitivity of a healthy Singaporean population. The test was also established as the preferred method for determination of detection threshold over GC-O dilution analysis for the ten odourants.

The Specific Sensitivity Test successfully evaluated the olfactory abilities of 281 adults from 21 to 80 years old. Loss in olfactory functions with age for single odourants was found to be in good agreement with normative data around the world for both odour mixtures and single odourants. Onset of decline in identification ability was from the 6th decade, whereas detection thresholds increased throughout adult lifetime and loss in sensitivity was not limited to the older age groups.

Heterogeneous losses in identification ability and threshold sensitivity of single odourants with age in the findings of the Specific Sensitivity Test were in agreement with the landmark National Geographic Smell Survey. Significantly, the Specific Sensitivity Test documented the varying extents and onset of olfactory sensitivity with age, demonstrating large differences in rates of loss with age. While the detection threshold of onion-like 2-methyl-3-tetrahydrofuran thiol was three times lower for subjects in their 20s than subjects in their 70s, the detection threshold for rose-like phenylethyl alcohol was almost 180 times lower in concentration for the same two groups of subjects. The varying extents of loss in threshold sensitivity of the ten odourants with age could not be explained by molecular weights, vapour pressure, boiling points, density, or molecular structure of the odourants. More odourants need to be tested to understand what factors affect the extents of odourant-specific olfactory loss with age.

The results of the Specific Sensitivity Test also demonstrated the age moderating effects on the relationship between identification ability and detection threshold of an odourant. Age moderation of odour identification and threshold sensitivity was especially pronounced in odourants with low

molecular weights. Further research needs to be completed in order to elucidate the mechanism for which olfactory training is able to improve identification proficiency and elevate detection thresholds.

Findings of the Specific Sensitivity Test were applied into flavours by the flavour modification method in order to evaluate if the technique is able to compensate for distortions in flavour perception as a result of heterogeneous age-associated olfactory loss in the elderly. However, the method was not found to be effective in this study, likely as a result of preconceived concepts of the flavours by the elderly.

Volatiles from food and beverages form odour perception in consumers via orthonasal and retronasal routes to the nasal cavity. As the Specific Sensitivity Test evaluated the orthonasal olfactory sensitivity of subjects with age, age-associated changes in retronasal olfactory sensitivity may be vastly different. Thus, further research in age-associated changes in retronasal olfactory testing will provide a clearer picture for the application of findings in flavour modification.

The Specific Sensitivity Test is the first portable olfactory test using only single odourants for both determination of identification ability and detection threshold sensitivity. Even though the Specific Sensitivity Test was developed with odourants familiar to the ethnic Chinese population in Singapore, the test may be applied to ethnic Indians and Malays to determine if the observed heterogeneous olfactory losses with age are affected by cultural and physiological differences. The Specific Sensitivity Test is a validated and reproducible olfactory test using single odourants commonly

found in natural foods. Therefore, the test may also be utilized and adapted for diagnostic tests of olfactory functions in populations beyond Singapore.

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APPENDICES

Appendix 1. Preliminary Survey for Food-Associated Odours Familiarity

Food Aromas - Do you know them? **Exit this survey**

1. Personal Information

1. What is your gender?

- Male
- Female

***2. Are you Singaporean/Singaporean PR?**

- Yes
- No

***3. How old are you?**

- 20 and below
- 21-30
- 31-40
- 41-50
- 51-60
- 61-70
- 71-80
- 81 and above

4. Are you a smoker?

- Yes
- No

5. What do you think of your sense of smell?

- Very poor
- Poor
- Average
- Good
- Very good

2. Food Aromas

Are you familiar with these smells?

***1. Please rate how well you know these smells.**
(1 = Not familiar at all; 5 = Know the smell very well)

	1	2	3	4	5
Butter	○	○	○	○	○
Pineapple	○	○	○	○	○
Garlic	○	○	○	○	○

Coffee					
Wine					
Jasmine					
Cedar					
Mango					
Pine					
Cabbage					
Lavender					
Fish					
Nutmeg					
Raspberry					
Pumpkin					
Papaya					
Bread					
Pepper					
Orange					
Coconut					
Pizza					
Almond					
Clove					
Peppermint					
Pear					
Chrysanthemum					
Mint					
Rootbeer					
Malt					
Cucumber					
Watermelon					
Walnut					
Soy					
Onion					
Vinegar					

Grapefruit					
Honey					
Lychee					
Anise					
Blackberry					
Lilac					
Milk					
Cherry					
Mustard					
Red Tea					
Lime					
Wood					
Caramel					
Banana					
Licorice					
Rose					
Chives					
Bubblegum					
Pickle					
Apple					
Chocolate					
Lemon					
Hazelnut					
Peach					
Cheddar cheese					
Peanut					
Melon					
Cinnamon					
Popcorn					
Gingerbread					
Grass					
Wintergreen					

Green Tea					
Sesame oil					
Durian					
Smoke					
Tangerine					
Potato					
Grape					
Strawberry					

3. Thank you

Thank you for your time!

Appendix 2. Preliminary Survey for Top 5 Favourite Flavours (English)

Name: _____ Age: _____

Gender: F / M

Please select your top 5 favourite flavours:

1. Almond
2. Banana
3. Bubblegum
4. Cherry
5. Coffee
6. Hazelnut
7. Lemon
8. Lychee
9. Mint
10. Pandan
11. Pear
12. Rose
13. Vanilla
14. Apple
15. Barbeque
16. Caramel
17. Chocolate
18. Grape
19. Honey
20. Jasmine
21. Mango
22. Orange
23. Peach
24. Pineapple
25. Strawberry

Appendix 3. Preliminary Survey for Top 5 Favourite Flavours (Chinese)

姓名: _____ 年龄: _____

性别: 男/女

请选择您最喜欢的五种口味:

1. 杏仁
2. 苹果
3. 香蕉
4. 烧烤
5. 泡泡糖
6. 焦糖
7. 樱桃
8. 巧克力
9. 咖啡
10. 葡萄
11. 榛子
12. 蜂蜜
13. 柠檬
14. 茉莉花
15. 荔枝
16. 芒果
17. 薄荷
18. 橙
19. 香兰
20. 桃
21. 梨
22. 菠萝
23. 玫瑰
24. 草莓
25. 香草